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Departamento de Biologia

**Mariana Oliveira Rodrigues**

# Impactos dos microplásticos em sistemas de água doce.

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Toxicologia e Ecotoxicologia, realizada sob a orientação científica da Doutora Ana Marta dos Santos Mendes Gonçalves, Investigadora do MARE do Departamento das Ciências da Vida da Universidade de Coimbra e do Departamento de Biologia & CESAM da Universidade de Aveiro, do Doutor Fernando José Mendes Gonçalves, Professor Associado com Agregação do Departamento de Biologia & CESAM, da Universidade de Aveiro e do Doutor Nelson José Cabaços Abrantes, Investigador auxiliar do Departamento de Ambiente e Ordenamento & CESAM da Universidade de Aveiro.

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Dedico aos meus pais, Amélia e António, e à minha avó, Maria Emília

*“Mais importante que correr atrás de um sonho é nunca desistir dele.”*

Autor desconhecido

**o júri**

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**palavras-chave**

Microplásticos; sistemas de água doce; metodologias de separação; contaminação.

**resumo**

Os plásticos, em particular os microplásticos (partículas com dimensões <5 mm), são poluentes ubíquos e persistentes que constituem uma preocupação científica e social emergente. As suas características, aliadas a uma gestão inadequada, contribuíram para a sua acumulação nos sistemas aquáticos, podendo atingir elevadas densidades. Estas partículas podem interagir com o ambiente, afetando a economia, a saúde humana e a estética. No entanto, a maioria dos estudos científicos tem-se focado no ambiente marinho, sendo o conhecimento sobre os sistemas de água doce escasso, incluindo em Portugal. Deste modo, este trabalho pretende contribuir para esta lacuna de informação, tanto ao nível da uniformização de metodologias de isolamento de microplásticos (MPs) em amostras de água como na caracterização de MPs num sistema de água doce português. Assim, numa primeira fase este estudo pretendeu avaliar a eficácia de diferentes métodos de separação, incluindo métodos de separação por densidade (açúcar, azeite e cloreto de zinco), bem como métodos de degradação de matéria orgânica (peróxido de hidrogénio e detergente multienzimático). Neste sentido, amostras artificiais contendo onze tipos de plásticos pertencentes aos polímeros mais comuns foram preparadas e submetidas aos diferentes métodos, procedendo-se posteriormente à quantificação e identificação dos polímeros usando um microscópio estereoscópico e um espectroscópio de infravermelhos com transformada de Fourier (FTIR). De entre os vários métodos testados, aquele que revelou o melhor custo-eficácia foi o método da oxidação com peróxido de hidrogénio e adição de cloreto de zinco. Este estudo enfatiza a importância do uso do cloreto de zinco tanto no processamento de amostras de sedimento como de água. Numa fase seguinte do estudo, determinou-se a abundância e distribuição de MPs na água e sedimento do rio Antuã, aplicando o método de separação identificado como o mais eficaz anteriormente. A abundância de MPs nas amostras de água variou entre 5 – 8.3 mg $m^{-3}$ ou 58 – 193 items $m^{-3}$ em Março e entre 5.8 – 51.7 mg $m^{-3}$ ou 71 – 1265 items $m^{-3}$ em Outubro. No sedimento, a abundância de MPs variou entre 13.5 – 52.7 mg $kg^{-1}$ ou 100 – 629 items $kg^{-1}$ em Março e entre 2.6 – 71.4 mg $kg^{-1}$ ou 18 – 514 items $kg^{-1}$ em Outubro. Estes resultados demonstram que este rio está severamente impactado por MPs, com valores semelhantes aos encontrados em sistemas marinhos/costeiros. Foi ainda observada uma variação espacial e temporal, dependente da estação do ano, do caudal do rio e da pressão antropogénica. Deste modo, este estudo vem enfatizar a importância dos rios como sistemas de transporte de MPs e realçar os potenciais impactos dos MPs como contaminantes emergentes nos ecossistemas aquáticos dulçaquícolas.

**keywords** Microplastics; freshwater systems; separation methods; contamination.

**abstract**

Plastics, in particular microplastics (particles with dimensions < 5 mm), are a widespread and persistent pollutant constituting an emerging scientific and societal issue. Its characteristics allied to an inadequate management contributes to their accumulation in aquatic systems, reaching high densities. Moreover, they can also interact with environment affecting economy, human health and aesthetics. However, most of scientific studies have been focused in marine environment while scarce knowledge exists regarding freshwater systems, including in Portugal. Hence, this study aimed to contribute to fill this gap of information both in uniformization of methodologies of isolation of microplastics (MPs) in water samples as well as on the MPs' characterization in a Portuguese freshwater system. Thus, the first part of this study aimed to assess the effectiveness of distinct separation methods including density separation methods (sucrose, olive oil and zinc chloride) as well as organic matter degradation methods (hydrogen peroxide and multienzymatic detergent). For that, artificial samples containing the eleven most common types of plastics were prepared, subjected to the different methods and then polymers were detected, quantified and identified using a stereoscope microscope and Fourier transform infrared spectroscopy (FTIR). Among the several tested methods, the most cost-effective was the method of wet peroxide oxidation with addition of zinc chloride. Hence, this study highlights the importance of the use of zinc chloride both in the processing of sediment and water samples. In a following step, the abundance and distribution of MPs in the water and sediment of Antuã river were determined by applying the separation method identified as the most effective previously. The abundance of MPs in water varied from 5 – 8.3 mg $m^{-3}$ or 58 – 193 items $m^{-3}$ in March and from 5.8 – 51.7 mg $m^{-3}$ or 71 – 1265 items $m^{-3}$ in October. In sediments, the abundance of MPs varied from 13.5 – 52.7 mg $kg^{-1}$ or 100 – 629 items $kg^{-1}$ in March and from 2.6 – 71.4 mg $kg^{-1}$ or 18 – 514 items $kg^{-1}$ in October. It shows that this river is severely impacted by MPs, in orders similar to that found in marine/coastal environments. A spatial and temporal variation was observed dependent on seasonal conditions, flow velocity and anthropogenic pressure. Thus, this study emphasizes the importance of rivers as carriage systems of MPs, and highlight the potential impacts of MPs as emerging contaminants on freshwater systems.

## Contents

## Abbreviations

| | |
|---|---|
| STP | Sewage treatment plant |
| ATR | Attenuated total reflection |
| $CaCl_2$ | Calcium chloride |
| CO | Carbonyl functional group |
| $C_xH_y$ | Organic substrates |
| ED | Enzymatic digestion |
| EDCs | Endocrine-disrupting substances |
| EVA | Ethylene-vinyl acetate |
| Fe (II) | Iron II |
| FTIR | Fourier transform infrared spectroscopy |
| HCl | Hydrochloric acid |
| HDPE | High density polyethylene |
| $H_2O_2$ | Hydrogen peroxide |
| HOCs | Hydrophobic organic chemicals |
| $KHCO_3$ | Potassium bicarbonate |
| LDPE | Low density polyethylene |
| L-MPP | Large microplastics |
| $MgCl_2.6H_2O$ | Magnesium chloride hexahydratade |
| MPs | Microplastics |
| MSFD | Marine Strategy Frame Work Directive |
| NaCl | Sodium chloride |
| $NaHCO_3$ | Sodium bicarbonate |
| NaI | Sodium iodide |
| NaOH | Sodium hydroxide |
| $Na_2SO_4$ | Sodium sulphate |
| N.I. | Not identified |
| nMDS | Non-metric multidimensional scaling |
| NT | No treatment |
| OH | Hydroxyl functional group |
| O.M. | Organic matter |

| | |
|---|---|
| OO | Olive oil |
| PA | Polyamide |
| PAHs | Polycyclic aromatic hydrocarbons |
| PBDEs | Polybrominated diphenyl ethers |
| PBTs | Persistent, bioaccumulative and toxic substances |
| PCBs | Polychlorinated biphenyls |
| PE | Polyethylene |
| PEA | Poly(ethylacrylate) |
| PES | Polyethersulfone |
| PET | Polyethylene terephthalate |
| PMMA | Polymethylmethaacrylate |
| PP | Polypropylene |
| PPCPs | Pharmaceutical and personal care products |
| PS | Polystyrene |
| PTFE | Polytetrafluorethylene |
| PUR | Polyurethane |
| PVA | Polyvinyl acetate |
| PVC | Polyvinyl chloride |
| Q | Final product obtained after oxidation process |
| RIC | Resin identification code |
| S | Sugar |
| S1 – S6 | Sediment samples from sites 1 to 3 in two different months |
| SBR | Butadiene/styrene copolymer |
| SDS | Sodium dodecylsulfate |
| S-MPP | Small microplastics |
| SPT | Sodium polytungstate |
| W1 – W6 | Water samples from sites 1 to 3 in two different months |
| WPO | Wet peroxide oxidation |
| WWT | Wastewater treatment |
| $ZnCl_2$ | Zinc chloride |

# Chapter 1
# General introduction

# Chapter 1 – General introduction

## 1. Plastics: a current problem

Fifty-four percent of the global mass of anthropogenic waste consist of plastic (Hoellein et al., 2014). Plastics are formed by long polymer chains and produced synthetically from organic products (e.g. hydrocarbon chains of distilled crude oil, cellulose, salt, coal or natural gas) with addition of various chemicals additives (e.g. plasticizers Bisphenol A and phthalates) (Andersson, 2014; Mintenig, 2014; Oliveira et al., 2013; Thompson et al., 2009). Due to its combined properties, such as lightness, inexpensive, versatility, durability, resistance and strength (Thompson et al., 2009), they are widely used in many industries (Andrady, 2011) and applications of almost every sector of our everyday life (Dris et al., 2015b). The diversity of polymers (e.g. low/high-density polyethylene, polypropylene, polystyrene, polyethylene terephthalate) and properties facilitates the production of a vast array of plastic products that bring remarkable societal benefits (e.g. technological advances and energy savings) (Andrady and Neal, 2009). The main markets that use plastics are packaging, building and construction. Nevertheless, plastics are also used in other sectors like household appliances, furniture, sport, health and safety. Since the middle of the 20$^{th}$ century, the worldwide production of plastic has increased over 100-fold (last data (2015): ≈ 322 million tonnes/year), with China as the largest producer. However, the production in Europe has been relatively constant in the last 10 years (last data (2015): ≈ 58 million tonnes/year) (PlasticsEurope, 2016). It is estimated that in 2050 this could reach the 33 billion tonnes of plastic (Rochman et al., 2013a). Although recycling and reuse of plastics are options that have increased, landfilling is still the first option in many EU countries, with almost 8 million tonnes of plastics waste landfilled in Europe. High production coupled with physical characteristics (chemical inertness and slow biodegradation), improper waste disposal (e.g. industry, urban waste, sewage treatment plant – STP, agriculture, accidental) and insufficient waste management (STP and Wastewater treatment - WWT) have resulted in an accumulation of plastic debris in the environment (Barnes et al., 2009; Dris et al., 2015b; Eubeler et al.,

2010; Thompson et al., 2004; Urgert, 2015). Once released in the environment, this material can be transported to aquatic ecosystems, in particular to freshwater systems, by direct inputs from the main sources of contamination, wind (atmospheric inputs) or rainy events, that washed the plastics from land to surface waters (Duis and Coors, 2016; Epa, 1992; Lambert et al., 2014; Ryan et al., 2009). This contamination not only includes plastic debris with large size (macroplastics) but also microplastics (Dris et al., 2015b) (Figure 1) that accumulate in water column or in sediments (acts like a reservoir of particles) (Castañeda et al., 2014; Free et al., 2014; Imhof et al., 2013; Zbyszewski et al., 2014). Currently, microplastics (MPs) represent contaminants of emerging scientific and societal concern (Bergmann et al., 2015; Wagner et al., 2014).

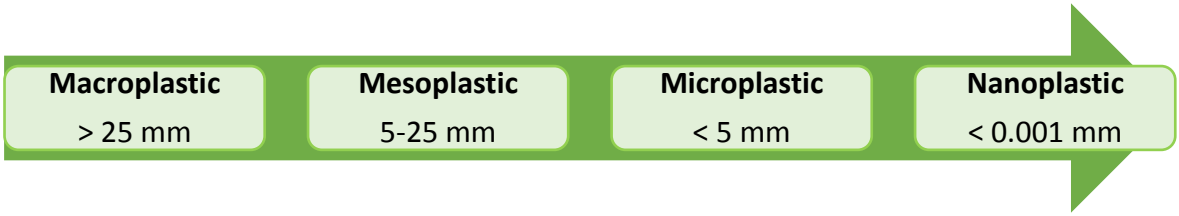


**Figure 1 –** Plastic debris nomenclature based on size suggested by European Marine Strategy Framework Directive (MSFD) technical subgroup on Marine Litter (MSFD GES Technical Subgroup on Marine Litter, 2013).

However, this contamination has been more investigated in marine environment (85.03%) than in freshwater lakes and rivers (14.97%) (Dris et al., 2015b; Wagner et al., 2014) (between 2004-2017). Nevertheless, publications are increasing, mostly since 2014. These studies reported MP contamination of lakeshore and riverbank sediment samples from continental freshwater systems in Europe (e.g. Seine and Rhine River; Lake Geneva, Constance), both North and South America (e.g. Elqui and Chicago River; Laurentian Great Lakes; Lake Winnipeg), Africa (Lake Victoria) and Asia (e.g. Lake Hovsgol and Taihu; Beijiang River) (Anderson et al., 2017; Besseling et al., 2013; Biginagwa et al., 2016; Dris et al., 2015a; Eriksen et al., 2013a; Faure et al., 2015; Mani et al., 2015; McCormick et al., 2014; Rech et al., 2014; Su et al., 2016; Wang et al., 2017). The concentration of MPs detected in these water systems are dependent on the proximity of source of contamination, ranging from 20,264 to 6 698,264 particles $km^{-2}$ (similar to those

found in marine systems) (Free et al., 2014; McCormick et al., 2014). In addition, for several authors rivers are being seen as carriage systems of MPs from terrestrial to marine environment (between 70-80%) (Eerkes-Medrano et al., 2015; GESAMP, 2010; Hidalgo-ruz et al., 2012; Klein et al., 2015; Urgert, 2015; Wagner et al., 2014).

This increasing concern promote integrated management of freshwaters and marine waters (policy initiatives and creation of legislation – *see* Figure 2) by European Union's Water Framework Directive (Directive 2000/60/EC of the European parliament and of the council of 23 October, 2000) and the Marine Strategy Framework Directive (Directive 2008/56/EC of the European parliament and of the council of 17 June, 2008). Furthermore, several activities and awareness actions have been developed to sensitize young people as well as general society (e.g. projects and campaigns: http://www.marliscoportugal.org/; http://www.5gyres.org/; https://www.youtube.com/watch?v=73sGgmZoMBQ; https://www.youtube.com/watch?v=Pt6KlPCX1BU). In 2011, 47 plastics associations across the globe have launched the Global Declaration for Solutions on Marine Litter and are supporting projects in six keys areas (education, research, public policy, sharing best practices, plastics recycling/recovery and plastic pellet containment). Despite of being projects/activities directed to marine ecosystems, they also should be applied in freshwater systems. Moreover, in recent years, "biopolymers" (e.g. starch-based polymers and polyesters – PCL and PLA) that could be obtained from biological and renewable resources (grains, corn, potatoes, beet sugar, sugar cane or vegetable oils) and are susceptible to recycle and biodegradation (microbial degradation into water, carbon dioxide or methane) have gather increasing attention (Gandini and Lacerda, 2015). "Biopolymers" have physical, optical and mechanical proprieties comparable to those of the traditional polymers (e.g. PET or PS) (da Costa et al., 2016). Since the plastics production is expected to continue on a positive trend, these "biopolymers" begin to be seen as good alternatives.

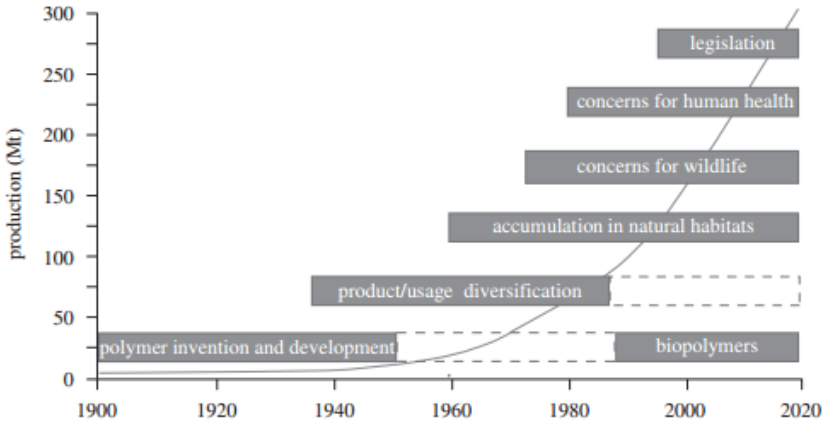


**Figure 2 –** Hindsight of the environmental concerns resulting from the plastic use. Taken from Thompson *et al.*(2009).

## 2. Characterization of microplastics and its detection

MPs are a very heterogeneous group of particles differing in morphological (size, shape, colour) (Desforges et al., 2014; Fries et al., 2013; Ivar do Sul et al., 2013; Vianello et al., 2013) and chemical characteristics (specific density and polymer type) (Rocha-Santos and Duarte, 2015), according to their source(s) (Duis and Coors, 2016). The current definition describes MPs as very small fragments of plastic with less than 5 mm (Arthur et al., 2009; Hidalgo-ruz et al., 2012), although some authors also subdivided into large (L-MPP: 1 – 5 mm) and small (S-MPP: 1 µm – 1 mm) MPs (Eriksen et al., 2013a, 2013b; Imhof et al., 2012). The lower size limit has been different among the studies, according to sample collection and analysis (Arthur and Baker, 2011; Duis and Coors, 2016; Faure et al., 2012). Besides the size, MPs can also be divided in types/groups according to their shape and colour, such as filament / fiber (thin or fibrous, straight plastic), film (thin plane of flimsy plastic), fragments (rounded, angular, hard and/or jagged plastic particle), pellets (cylinders, disks, spherules opaque and/or transparent, hard plastic particle) and foam (lightweight, sponge-like plastic) (Figure 3) (Free et al., 2014; Mani et al., 2015; Wagner et al., 2014). These characteristics are visual and well defined for known polymers, however can lead to erroneous characterization of microparticles (Lee et al., 2014).

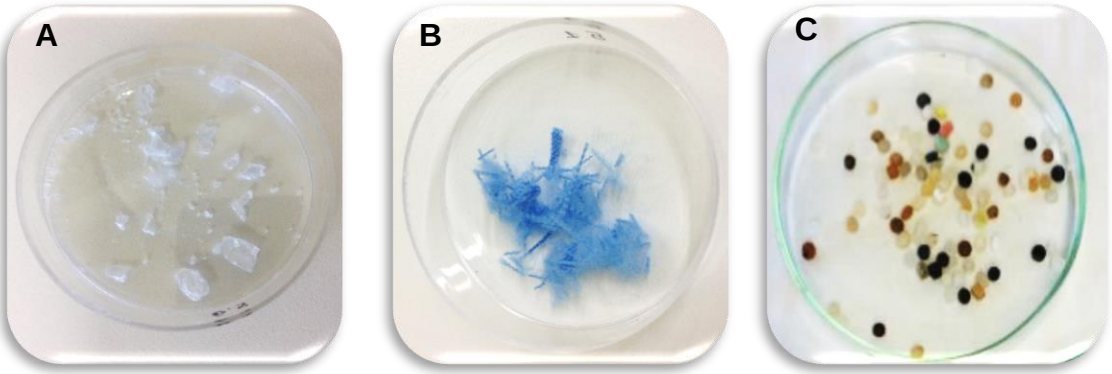


**Figure 3 –** Three types of MPs according to their shape: A – Fragments; B – Fibers; C – Pellets.

Each polymer also has a specific density (Table 1) that conditionate the place they will occupy in the water system. Among these polymers, the most widely produced ones are high- and low-density polyethylene (HD/LD-PE), polypropylene (PP), polyethylene terephthalate (PET), polyvinylchloride (PVC), polyurethane (PUR) and polystyrene (PS) (Table 1) (PlasticsEurope, 2016; Rocha-Santos and Duarte, 2015; Wagner et al., 2014).

**Table 1** – Densities and examples of applications of common plastic types, at room temperature. Adapted from da Costa et al. (2017), Driedger et al. (2015), Mintenig (2014), and PlasticsEurope (2016).

| Polymer type | Abbreviation | Applications (e.g.) | Density (g $cm^{-3}$) |
|---|---|---|---|
| Low-density Polyethylene | LDPE | Packaging, general purpose containers, agricultural film | 0.89 – 0.93 |
| High-density Polyethylene | HDPE | Milk containers, detergent bottles, tubing | 0.94 – 0.98 |
| Polypropylene | PP | Packaging, bottle caps, ropes, carpets, laboratory equipment, drinking straws | 0.85 – 0.92 |
| Polystyrene | PS | Packaging foam, disposable cups, CDs, food containers | 1.04 – 1.09 |
| Polyamide (nylon) | PA | Textiles, toothbrush bristles, fishing lines, automotive | 1.02 – 1.05 |

| | | | |
|---|---|---|---|
| Polyvinyl chloride | PVC | Pipes, window frames, flooring, shower curtains | 1.16 – 1.58 |
| Polymethyl methacrylate (acrylic) | PMMA | Touch screens | 1.16 – 1.20 |
| Polyurethane | PUR | Building insulation, pillows and mattresses, insulating foams for fridges | 1.2 |
| Polyethersulfone | PES | Textiles | 1.24 – 2.30 |
| Polyethylene terephthalate (polyester) | PET | Soft drink bottles, food packaging, thermal insulation, blister packs | 1.37 – 1.45 |

According to their origin, these fragments can be further classified into primary and/or secondary MPs. Their source will determine their shape, chemical composition and surface features (Blair et al., 2017). On one hand, primary MPs are defined as MPs produced in a micro-size range for direct use or as precursors to other products (Duis and Coors, 2016; Mintenig, 2014). Specific personal care products (e.g. exfoliants and/or abrasives) and medical applications (e.g. dentist tooth polish) containing MPs, drilling fluids for oil and gas exploration, industrial abrasives, improper handling and pre-production plastics (accidental losses, runoff from processing facilities) are the main sources of primary MPs (Duis and Coors, 2016). On the other hand, secondary MPs resulting from the continuous fragmentation of macroplastics caused by a combination of abiotic and biotic mechanisms (photodegradation, mechanical abrasion, chemical, biological and thermal degradation and disintegration) (Beyler and Hirschler, 2001; Cole et al., 2011; Eubeler et al., 2010; Lucas et al., 2008). These mechanisms depend on the environmental settings and the physical and chemical characteristics of the polymeric materials (Lambert et al., 2014). Exposure to visible (400 – 700 nm) and high-energy UV radiation (290 - 400 nm) can cause photodegradation that is, generally, considered to be the most efficient abiotic degradation route occurring in the environment, in particular in freshwater systems (da Costa et al., 2017; Horton et al., 2017). Plastics can absorb this radiation, leading to a higher reactivity of their electrons, which induces oxidation and cleavage. These degradation processes are

mediated by chain scission and cross-linking reactions (Kaczmarek et al., 2007; Lucas et al., 2008). Oxidation derives from the introduction of oxygen into the polymer chain and leads to the formation of carbonyl (CO) and hydroxyl (OH) functional groups. Since abiotic degradation processes cause mechanical and structural changes (e.g. larger surface areas), the formation of these groups contributes to subsequent biotic degradation routes (da Costa et al., 2017). The macroplastics that give origin to this type of MP are from plastic waste dumped, losses of waste during waste collection (landfill sites and recycling facilities), synthetic polymer particles used to improve soil quality, abrasion/release of fibers from synthetic textiles, hygiene products and paints based on synthetic polymers (e.g. ship paints, house paint, road paint). There are some MPs that could be regarded either as primary or secondary (e.g. fibers from synthetic clothes). In most cases, it is not possible to derive assumptions on the origin of MPs, except for those with a typical and distinct size and shape (Duis and Coors, 2016).

Given that plastic is environmental persistent its detection in freshwater systems depends on physical and temporal factors. According to Simpson *et al*. (2005) and Rocha-Santos and Duarte (2015), the physical factors (hydrodynamic and geographical conditions) that might influence the particle transport in freshwater are flow velocity, turbidity, turbulence, density of water mass, water depth, substrate type, bottom topography, and seasonal variability of water flows. Other factors, as storms, floods or anthropogenic activities (littering or recycling) might also influence temporal detection (Kessarkar et al., 2010; Moatar et al., 2006). These factors combined with particle characteristics (size, density and composition) can determine the behaviour of particles in aquatic systems and can improve understanding of the transport dynamics and the accumulation zones. These factors are similar to those that affect sediment transport (Nizzetto et al., 2016). For example, MPs with lower density than freshwater ($\approx$ 1,0 g $cm^{-3}$) are buoyant (occupy the pelagic transport route in suspension) and those with higher density are submerged (occupy benthic transport route as bed load) (Eisma and Cadeé, 1991; Imhof et al., 2012). The density can be changed by the colonisation of organisms in the surface of MPs and erosion of MPs' surface (by biotic and abiotic processes) (da Costa et al., 2016; Goldstein et al., 2013).

## 3. Impacts of microplastics on aquatic systems

### 3.1 Effects on biotic and abiotic environment

MPs may have negative impacts on aquatic systems, in particular on aquatic organisms and on its abiotic factors (Figure 4). Although the majority of studies focuses the impacts of MPs in marine organisms (e.g. Besseling et al., 2013; Brillant and MacDonald, 2000; Browne et al., 2008; Cole et al., 2013; Farrell and Nelson, 2013; Graham and Thompson, 2009; Lusher et al., 2013; Murray and Cowie, 2011; Rochman et al., 2013b; Setälä et al., 2014; Tanaka et al., 2013; Van Cauwenberghe and Janssen, 2014; von Moos et al., 2012; Wright et al., 2013), only few studies report impacts in freshwater organisms. Concerning the studies on freshwater systems, they have demonstrated through laboratory assays that organisms, such as fishes (e.g. *Gobio gobio*, *Platichthys flesus*, *Pomatoschistus microps, Oryzias latipes* and *Osmerus eperlanus*), annelids (e.g. *Lumbriculus variegatus*), ostracods (e.g. *Notodromas monacha*), gastropods (e.g. *Potamopyrgus antipodarum*), crustaceans (e.g. *Gammarus fossarum*, *Gammarus pulex*, *Hyalella azteca* and *Daphnia magna*) and birds may ingest MPs (Au et al., 2015; Blarer and Burkhardt-Holm, 2016; Eerkes-Medrano et al., 2015; Imhof et al., 2013; Jemec et al., 2016; McGoran et al., 2017; Oliveira et al., 2013; Rochman et al., 2013b; Rosenkranz et al., 2009; Sanchez et al., 2014).This ingestion is influenced by particle size, with smaller particles having higher potential to be ingested by a greater number of organisms (Horton et al., 2017), but also particle shape, density and colour (Shaw and Day, 1994). After ingestion, MPs can remain in the digestion tract, be excreted within hours or in few days, or translocated from the digestive tract into the body tissue (e.g. intestinal tract, testis, liver, brain) and consequently into fluids (e.g. circulatory system or lipid storage droplets) (Dris et al., 2015b; Eerkes-Medrano et al., 2015). If excretion is absent or inefficient, MPs will cause adverse effects in organism's performance (physical impacts), such as, blocked digestive tracts (accumulation of MPs), lacerations, inflammatory responses, reduced rates of respiration, false sense of satiation, impaired feeding capacity, starvation, debilitation, limited predator avoidance, early tumour formation or death/immobilisation. These physical effects can lead to a significant decrease in

growth, fitness and reproduction, as reported by Au *et al*. (2015) and Blarer and Burkhardt-Holm (2016), after exposure of *Hyalella azteca* and *Gammarus fossarum* to MPs. This reduction may be explained by the increment of residence time in the gut that might affect the ability to assimilate and process food, resulting in an energetic effect (decrease in survival, growth rates and reproduction and increase on organism's development) (Maltby, 1994; Wright et al., 2013). de Sá *et al.*(2015) and Galloway et al. (2017) added to the reduced ability of the organisms to discriminate MPs from the real prey. Other study, suggest that earlier fish stages (e.g. embryos) are more sensitive to MP exposure than later stages (e.g. juvenile fish), which could have consequences for juvenile growth rates or survival (Eerkes-Medrano et al., 2015; Rochman et al., 2013a).

At the molecular and cellular levels, the ingestion of MPs can induce, in Medaka fish (*Oryzias latipes*) and Zebrafish (*Danio rerio*), liver stress response/inflammatory responses, resulting in glycogen depletion, fatty vacuolation and single cell necrosis (Lu et al., 2016; Rochman et al., 2013b). Furthermore, lipid metabolites of triglycerides, fatty acids (monounsaturated fatty acid – MUFA, linoleic acid, FA-$\alpha H_2$, FA-$\omega$-$CH_3$ and fatty acyl chains), choline, phosphorycoline, cholesterol and branched-chain amino acids (BCAAs – isoleucine, valine and leucine) can be changed, indicating that MPs can induce a disruption of lipid metabolism (in zebrafish). BCAAs can promote fatty acids metabolism, prevent fat accumulation and also regulate energy metabolism, that will decrease after exposure to MPs (Lu et al., 2016). Moreover, this author observed and increase in superoxide dismutase (SOD) and catalase (CAT) activities, indicating an oxidative stress. According to Oliveira *et al.*(2013), MPs are also able to reduce significantly the acetylcholinesterase activity (by an average of 22%) of Goby fish (*Pomatoschistus microps*), however the levels of lipid peroxidation (LPO), lactate dehydrogenase (LDH) and glutathione S-transferase (GST) activity did not show changes (suggesting no involvement of this enzyme in their biotransformation or any oxidative damage). This inhibition is high enough to induce adverse effects in cholinergic neurotransmission and thus potentially in nervous and neuromuscular function (Ludke et al., 1975; Oliveira et al., 2013). Since MPs are widespread in aquatic systems and this enzyme has a pivotal role in neurological function of many

organisms, in particular in fishes, in the control of several physiological processes (e.g. growth and reproduction), and behavioural processes (e.g. swimming), this inhibition could be alarming due to the direct or indirectly influence at the individual and populational fitness.

Accumulation of MPs in freshwater sediments, in the water column and later ingestion by freshwater benthic and pelagic fauna, respectively, might have cascading effects with trophic (bioaccumulation in lower trophic levels, e.g. benthic invertebrates or planktonic organisms, and consequently in higher trophic levels, e.g. fishes and humans) and ecosystem consequences (e.g. impacts on community structure) (Eerkes-Medrano et al., 2015; Lechner et al., 2014; Schindler and Scheuerell, 2002). Many affected species are important to ecosystems processes such as decomposition and nutrient cycling (Horton et al., 2017).

The effects of MPs may also be transferred between habitats, from freshwater to terrestrial systems, since many freshwater organisms are prey to terrestrial insects, amphibians, reptiles and birds (Polis et al., 1997).

In addition, to have direct interactions with organisms, MPs may have wider impacts by interacting directly or indirectly with the abiotic characteristics of the environment (e.g. alterations in light penetration and in sediment characteristics, like pore and grain size, capacity to bind chemicals) that could affect biogeochemical cycles (Arthur and Baker, 2011; Simpson et al., 2005).

### 3.2 Microplastics as a vector of contaminants, exotic species and pathogens

Due to their large surface-to-volume ratio and chemical composition (hydrophobicity) (Cole et al., 2013), MPs may act as a medium/vector to concentrate and transfer chemicals and persistent, bioaccumulative and toxic substances (PBTs) to organisms (Ashton et al., 2010; Browne et al., 2013; Engler, 2012; Teuten et al., 2009, 2007). These PBTs could be hydrophobic organic chemicals (HOCs) that are adsorbed onto their surface from the environment such as metals, polybrominated diphenyl ethers (PBDEs), endocrine-disrupting substances (EDCs), pharmaceutical and personal care products (PPCPs), polychlorinated biphenyls

(PCBs), polycyclic aromatic hydrocarbons (PAHs) or dioxins. Moreover, they also could be chemicals that are added to the plastic (e.g. plasticizers, colourants, flame retardants) during the plastic production process (Besseling et al., 2013; Browne et al., 2013; Lithner et al., 2009; Mato et al., 2001; Moore, 2008; Talsness et al., 2009). In freshwater systems, the concentrations of HOCs are expected to be higher than in marine environment, due to the proximity of the use of these chemicals (Dris et al., 2015b). Chemical transfer depends on the plastic (e.g. polyethylene sorbs greater concentrations of contaminants than other polymers), the contaminant, the surrounding environment, and the organism that ingests the plastic (e.g. if organism has a warm-blood and a low pH facilitates the release of contaminants from plastics) (Bakir et al., 2014). Many of these chemicals added to plastics are weakly bound or not bound at all to the polymer molecule and after UV degradation and high temperatures they will be transferred to water, sediment and organisms (Andrady, 2011; Horton et al., 2017). Bisphenol-A, phthalates (e.g. di-n-butyl phthalate, di-(2-ethylhexyl) phthalate), PBDEs and metals (e.g. Cadmium), have been identified as either toxic or endocrine disruptors (Hu et al., 2005; Kim et al., 2006; Lithner et al., 2009; Oehlmann et al., 2009; Rochman et al., 2013b; Teuten et al., 2009). They are lipophilic, making them suitable for penetrating cell membranes and to subsequently participate in biochemical reactions, with severe behavioural and reproductive effects. PCBs, PAHs and PBDEs have been proven to mimic hormones inducing reproductive disorders. Moreover, they are very resistant to environmental degradation (biological, chemical and photolytic) and can be involved in bioaccumulation and bioamplification phenomena (da Costa et al., 2016). Studies show that MPs can modulate contaminant's toxicity and the mixture (MP + PBT) can induce liver stress (glycogen depletion and histopathological alterations) and higher metabolites accumulation in fishes (Oliveira et al., 2013; Rochman et al., 2013b).

MPs serve also as novel ecological habitats for several microorganisms such as microbial community that influence plastic debris degradation and may provide substrate for opportunistic (human) pathogens (Zettler et al., 2013), such as specific members of the genus *Vibrio* (Wagner et al., 2014). This last bacteria can impact the water quality for human supply. Their hydrophobic surface stimulates rapid biofilm formation (Zettler et al., 2013). Given that MPs can be transported over long

distances (due to the low size), they facilitate the dispersal of species (invasive species and exotic species) and pathogens (Zettler et al., 2013). In addition, they exhibit a longer half-life than other substrates, which will provide an interesting habitat for microbial biofilm formation but also for epiplastic organisms like diatoms, ciliates and other organisms that will attach to the formed biofilm (Dobretsov, 2010; Reisser et al., 2014).

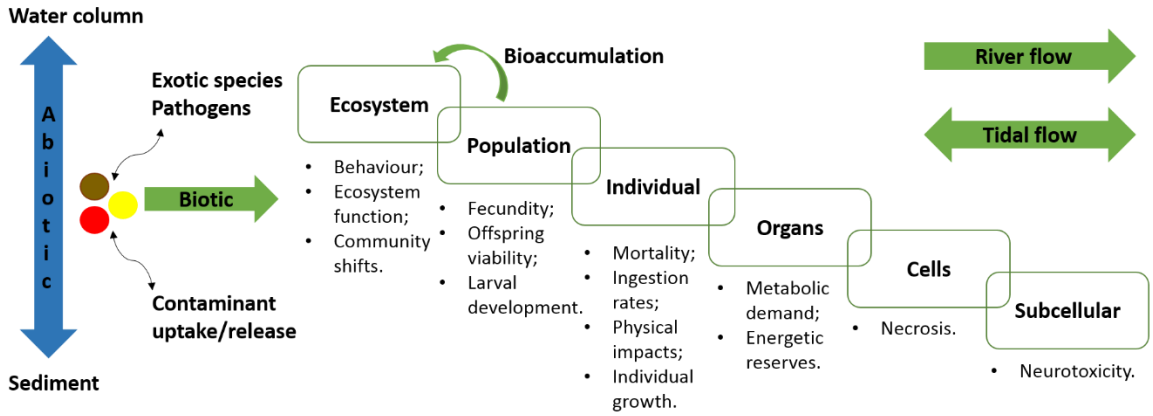


**Figure 4 –** Scheme illustrating potential impacts of exposure to MPs across successive levels of biological organization (from the highest ecological relevance– ecosystem – to the lowest – subcellular level).

## 4. Socio-economic implications

The presence and accumulation of MPs in freshwater environments may have the potential to exert direct or indirect effects in the economy sector. Water used for drinking, bathing, cooking and irrigating agricultural fields are from freshwater systems (Eerkes-Medrano et al., 2015). These systems are subjected to wastewater treatment (WWT) to remove particles and pollutants (e.g. MPs) (Carr et al., 2016). However, some authors reported that chemicals and MPs are present in water entering treatment plants (Murphy et al., 2016), in treated effluent and in drinking water (Brausch and Rand, 2011; Morasch et al., 2010) putting risks to human health. If MPs are present in these ecosystems, opportunistic pathogens may also be present, affecting the water quality (Van Cauwenberghe et al., 2015; Wagner et al., 2014). The accumulation of MPs in freshwater systems can also

constitute a risk of contamination to local activities such as aquacultures and agriculture, with potential consequences for humans that could feed from contaminated products and for producers that will lose their entire stock. Besides the risk of contamination, MPs can damage equipment used in these activities. These outcomes have significant economic costs, sometimes intangibles that, in most cases, are not borne by polluters (da Costa et al., 2016).

Since water from the rivers is also used for fishing, its contamination by MPs and, in the worst case scenario, by PBTs, can lead to prohibition of fishing. Furthermore, MPs can damage propellers and others boat's equipment (Galgani et al., 2010). These prohibitions may also have social-economic impacts. Other socio-economic impacts are the decrease interest of tourists from polluted places, the risk of navigation in freshwater systems and the extra money that local authorities need to spend in cleaning and extra dredging (Galgani et al., 2010; Newman et al., 2015).

## 5. Current situation

### 5.1 Portugal

The evaluation of MPs contamination in freshwater systems are increasing worldwide, however there are no studies in Portuguese freshwater systems. In addition, there are only 3 publications about MPs in Portuguese marine environment (Frias et al., 2016, 2013; Martins and Sobral, 2011) and 3 about macroplastics (Frias et al., 2010; Martins, 2011; Sá et al., 2016). According these studies in marine environment, the concentration found in the Southern Portuguese shelf waters (Algarve) was 0.01 items $g^{-1}$ of sediment (Frias et al., 2016) and in beaches of Alcobaça municipality was on average 452 g $m^{-2}$ (Frias et al., 2013). In addition, Martins and Sobral (2011) found 133.3 items $m^{-2}$ in beach sediment along Portuguese coast. Since Portugal is the 12$^{th}$ country with the highest plastics demand (≈ 1 million tonnes) and 10% - 50% of plastic go to landfills (it is not banned) (PlasticsEurope, 2016), there is an extensive gap of information about the levels of MP contamination in water and sediment, both in freshwater and marine systems. Moreover, the recycling and landfill are the preferred options for plastics waste, followed by energy recovery.

## 6. Objectives

In order to reduce the gap of information both in the uniformization of methodologies for MPs isolation in water samples and, on the levels of MPs contamination in Portuguese freshwater systems, the present work aimed to:

1. Assess the effectiveness of distinct separation methods as an attempt to identify and establish an unified method;
2. Evaluate the MPs contamination of Antuã River, in water and sediment samples, from two different seasons (Spring and Autumn).

To perform the first objective, artificial freshwater samples containing eleven plastic products (e.g. low/high-density polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate) were prepared and subjected to 6 distinct methods, selected according to their common application and efficiency: density separation methods using sugar, olive oil and zinc chloride, as well as organic matter degradation methods with hydrogen peroxide (wet peroxide oxidation) and multienzymatic detergent (enzymatic digestion). The identification was based on cost-effective parameters: cost, density separation and organic matter degradation efficiency, total mass of recovered polymers, time spent, simplicity and quality of recovered polymers. The quality of polymers was guaranteed by Fourier transform infrared spectroscopy (FTIR) and a wet peroxide oxidation (WPO) experiment with fibers (Tetra Pak® packaging and fabric).

After identification of the most cost-effective method, water and sediment samples of 3 sampling locations along Antuã River from two different seasons (Spring and Autumn) were subjected to that method. They were then undergoing the detection, quantification and identification of polymers using a stereomicroscope and FTIR. Hence, this last study aimed to describe and characterize the distribution and abundance of MPs in Antuã River, to compare the results between seasons, to illustrate the transport and potential sources of plastic debris and to present the potential effects of abiotic factors on the degradation of MPs.

## 7. References


Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L., Rennie, M.D., 2017. Microplastic contamination in Lake Winnipeg, Canada. Environ. Pollut. 225, 223–231. doi:10.1016/j.envpol.2017.02.072

Andersson, E., 2014. Micro plastics in the oceans and their effect on the marine fauna. Fakulteten för veterinärmedicin och husdjursvetenskap.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. doi:10.1016/j.marpolbul.2011.05.030

Andrady, A.L., Neal, M. a, 2009. Applications and societal benefits of plastics. Philos. Trans. R. Soc. B Biol. Sci. 364, 1977–1984. doi:10.1098/rstb.2008.0304

Arthur, C., Baker, J., 2011. Proceedings of the Second Research Workshop on Microplastic Marine Debris. November 5-6, 2010. NOAA Technical Memorandum NOS-OR&R-39.

Arthur, C., Baker, J., Bamford, H., 2009. Proceedings of the International Research Workshop on the Occurrence , Effects , and Fate of Microplastic Marine Debris. Sept 9-11, 2008. NOAA Technical Memorandum NOS-OR&R-30.

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055. doi:10.1016/j.marpolbul.2010.07.014

Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of *Hyalella azteca* to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34, 2564–2572. doi:10.1002/etc.3093

Bakir, A., Rowland, S.J., Thompson, R.C., 2014. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ. Pollut. 185, 16–23. doi:10.1016/j.envpol.2013.10.007

Barnes, D.K. a, Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. 364, 1985–1998. doi:10.1098/rstb.2008.0205

Bergmann, M., Gutow, L., Klages, M., 2015. Marine Anthropogenic Litter. Springer International Publishing, Cham. doi:10.1007/978-3-319-16510-3

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans,

A.A., 2013. Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47, 593–600. doi:10.1021/es302763x

Beyler, C.L., Hirschler, M.M., 2001. Thermal Decomposition of Polymers, in: DiNenno, P.J. (Ed.), SPE Handbook of Fire Protection Engineering. pp. 110–131. doi:10.1021/cm200949v

Biginagwa, F.J., Mayoma, B.S., Shashoua, Y., Syberg, K., Khan, F.R., 2016. First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia. J. Great Lakes Res. 42, 146–149. doi:10.1016/j.jglr.2015.10.012

Blair, R.M., Waldron, S., Phoenix, V., Gauchotte-Lindsay, C., 2017. Micro- and Nanoplastic Pollution of Freshwater and Wastewater Treatment Systems. Springer Sci. Rev. doi:10.1007/s40362-017-0044-7

Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. Environ. Sci. Pollut. Res. 23, 23522–23532. doi:10.1007/s11356-016-7584-2

Brausch, J.M., Rand, G.M., 2011. A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. Chemosphere 82, 1518–1532. doi:10.1016/j.chemosphere.2010.11.018

Brillant, M.G.S., MacDonald, B.A., 2000. Postingestive selection in the sea scallop, *Placopecten magellanicus* (Gmelin): the role of particle size and density. J. Exp. Mar. Bio. Ecol. 253, 211–227. doi:10.1016/S0022-0981(00)00258-6

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested Microscopic Plastic Translocates to the Circulatory System of the Mussel, *Mytilus edulis* (L.). Environ. Sci. Technol. 42, 5026–5031. doi:10.1021/es800249a

Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic Moves Pollutants and Additives to Worms, Reducing Functions Linked to Health and Biodiversity. Curr. Biol. 23, 2388–2392. doi:10.1016/j.cub.2013.10.012

Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174–182.

doi:10.1016/j.watres.2016.01.002

Castañeda, R.A., Avlijas, S., Simard, M.A., Ricciardi, A., Smith, R., 2014. Microplastic pollution in St. Lawrence River sediments. Can. J. Fish. Aquat. Sci. 71, 1767–1771. doi:10.1139/cjfas-2014-0281

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic Ingestion by Zooplankton. Environ. Sci. Technol. 47, 6646–6655. doi:10.1021/es400663f

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull. 62, 2588–2597. doi:10.1016/j.marpolbul.2011.09.025

da Costa, J.P., Duarte, A.C., Rocha-Santos, T.A.P., 2017. Microplastics – Occurrence, Fate and Behaviour in the Environment, in: Comprehensive Analytical Chemistry. pp. 1–24. doi:10.1016/bs.coac.2016.10.004

da Costa, J.P., Santos, P.S.M., Duarte, A.C., Rocha-Santos, T., 2016. (Nano)plastics in the environment – Sources, fates and effects. Sci. Total Environ. 566–567, 15–26. doi:10.1016/j.scitotenv.2016.05.041

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. doi:10.1016/j.envpol.2014.10.026

Desforges, J.W., Galbraith, M., Dangerfield, N., Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. Mar. Pollut. Bull. 79, 94–99. doi:10.1016/j.marpolbul.2013.12.035

Directive 2000/60/EC of the European parliament and of the council of 23 October, 2000. Establishing a framework for Community action in the field of water policy (Water Framework Directive). Off. J. Eur. Communities L327, 1–72. doi:10.1039/ap9842100196

Directive 2008/56/EC of the European parliament and of the council of 17 June, 2008. Establishing a framework for Community action in the field of marine environmental policy (Marine Strategy Framework Directive). Off. J. Eur. Union L164, 19–40. doi:10.1016/j.biocon.2008.10.006

Dobretsov, S., 2010. Marine Biofilms, in: Durr, S., Thomason, J.C. (Eds.), Biofouling. Wiley-Blackwell, Oxford, UK, pp. 123–136. doi:10.1002/9781444315462.ch9

Driedger, A.G.J., Dürr, H.H., Mitchell, K., Van Cappellen, P., 2015. Plastic debris in the Laurentian Great Lakes: A review. J. Great Lakes Res. 41, 9–19. doi:10.1016/j.jglr.2014.12.020

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015a. Microplastic contamination in an urban area: a case study in Greater Paris. Environ. Chem. 12, 592. doi:10.1071/EN14167

Dris, R., Imhof, H., Sanchez, W., Gasperi, J., Galgani, F., Tassin, B., Laforsch, C., 2015b. Beyond the ocean: contamination of freshwater ecosystems with (micro-)plastic particles. Environ. Chem. 12, 539. doi:10.1071/EN14172

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28, 2. doi:10.1186/s12302-015-0069-y

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82. doi:10.1016/j.watres.2015.02.012

Eisma, D., Cadeé, G.C., 1991. Particulate Matter Processes in Estuaries, in: Degens, E.T., Kempe, S., Richey, J.E. (Eds.), Biogeochemistry of Major World Rivers. John Wiley and Sons Ltd, pp. 283–296.

Engler, R.E., 2012. The Complex Interaction between Marine Debris and Toxic Chemicals in the Ocean. Environ. Sci. Technol. 46, 12302–12315. doi:10.1021/es3027105

Epa, 1992. Plastic Pellets in the Aquatic Environment: Sources and Recommendations, United States Environmental Protection Agency. Office of Water, Washington DC. doi:EPA 842-S-93-001

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013a. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. 77, 177–182. doi:10.1016/j.marpolbul.2013.10.007

Eriksen, M., Maximenko, N., Thiel, M., Cummins, A., Lattin, G., Wilson, S., Hafner,

J., Zellers, A., Rifman, S., 2013b. Plastic pollution in the South Pacific subtropical gyre. Mar. Pollut. Bull. 68, 71–76. doi:10.1016/j.marpolbul.2012.12.021

Eubeler, J.P., Bernhard, M., Knepper, T.P., 2010. Environmental biodegradation of synthetic polymers II. Biodegradation of different polymer groups. TrAC Trends Anal. Chem. 29, 84–100. doi:10.1016/j.trac.2009.09.005

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. doi:10.1016/j.envpol.2013.01.046

Faure, F., Corbaz, M., Baecher, H., de Alencastro, L.F., 2012. Pollution due to plastics and microplastics in Lake Geneva and in the Mediterranean Sea. Arch. Sci. 65, 157–164.

Faure, F., Demars, C., Wieser, O., Kunz, M., de Alencastro, L.F., 2015. Plastic pollution in Swiss surface waters: nature and concentrations, interaction with pollutants. Environ. Chem. 12, 582. doi:10.1071/EN14218

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. 85, 156–163. doi:10.1016/j.marpolbul.2014.06.001

Frias, J.P.G.L., Antunes, J.C., Sobral, P., 2013. Local marine litter survey - A case study in Alcobaça municipality, Portugal. Rev. Gestão Costeira Integr. 13, 169–179. doi:10.5894/rgci395

Frias, J.P.G.L., Gago, J., Otero, V., Sobral, P., 2016. Microplastics in coastal sediments from Southern Portuguese shelf waters. Mar. Environ. Res. 114, 24–30. doi:10.1016/j.marenvres.2015.12.006

Frias, J.P.G.L., Sobral, P., Ferreira, A.M., 2010. Organic pollutants in microplastics from two beaches of the Portuguese coast. Mar. Pollut. Bull. 60, 1988–1992. doi:10.1016/j.marpolbul.2010.07.030

Fries, E., Dekiff, J.H., Willmeyer, J., Nuelle, M.-T., Ebert, M., Remy, D., 2013. Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. Environ. Sci. Process. Impacts 15, 1949. doi:10.1039/c3em00214d

Galgani, F., Fleet, D., Van Franeker, J., Katsanevakis, S., Maes, T., Mouat, J.,

Oosterbaan, L., Poitou, I., Hanke, G., Thompson, R., Amato, E., Birkun, A., Janssen, C., 2010. Marine Strategy Framework Directive: Task Group 10 Report Marine Litter, Luxembourg: Office for Official Publications of the European Communities. doi:10.2788/86941

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1, 116. doi:10.1038/s41559-017-0116

Gandini, A., Lacerda, T.M., 2015. From monomers to polymers from renewable resources: Recent advances. Prog. Polym. Sci. 48, 1–39. doi:10.1016/j.progpolymsci.2014.11.002

GESAMP, 2010. Proceedings of the GESAMP International Workshop on micro-plastic particles as a vector in transporting persistent, bio-accumulating and toxic substances in the oceans., in: Bowmer, T., Kershaw, P. (Eds.), . p. 68. doi:10.1007/978-94-011-3610-5

Goldstein, M.C., Titmus, A.J., Ford, M., 2013. Scales of Spatial Heterogeneity of Plastic Marine Debris in the Northeast Pacific Ocean. PLoS One 8, e80020. doi:10.1371/journal.pone.0080020

Graham, E.R., Thompson, J.T., 2009. Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J. Exp. Mar. Bio. Ecol. 368, 22–29. doi:10.1016/j.jembe.2008.09.007

Hidalgo-ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environ. Sci. Technol. 46, 3060−3075. doi:dx.doi.org/10.1021/es2031505

Hoellein, T., Rojas, M., Pink, A., Gasior, J., Kelly, J., 2014. Anthropogenic Litter in Urban Freshwater Ecosystems: Distribution and Microbial Interactions. PLoS One 9, e98485. doi:10.1371/journal.pone.0098485

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141. doi:10.1016/j.scitotenv.2017.01.190

Hu, X., Wen, B., Zhang, S., Shan, X., 2005. Bioavailability of phthalate congeners

to earthworms (*Eisenia fetida*) in artificially contaminated soils. Ecotoxicol. Environ. Saf. 62, 26–34. doi:10.1016/j.ecoenv.2005.02.012

Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. Curr. Biol. 23, R867–R868. doi:10.1016/j.cub.2013.09.001

Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P., Laforsch, C., 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnol. Oceanogr. Methods 10, 524–537. doi:10.4319/lom.2012.10.524

Ivar do Sul, J.A., Costa, M.F., Barletta, M., Cysneiros, F.J.A., 2013. Pelagic microplastics around an archipelago of the Equatorial Atlantic. Mar. Pollut. Bull. 75, 305–309. doi:10.1016/j.marpolbul.2013.07.040

Jemec, A., Horvat, P., Kunej, U., Bele, M., Kržan, A., 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean *Daphnia magna*. Environ. Pollut. 219, 201–209. doi:10.1016/j.envpol.2016.10.037

Kaczmarek, H., Bajer, K., Gałka, P., Kotnowska, B., 2007. Photodegradation studies of novel biodegradable blends based on poly(ethylene oxide) and pectin. Polym. Degrad. Stab. 92, 2058–2069. doi:10.1016/j.polymdegradstab.2007.07.019

Kessarkar, P.M., Purnachandra Rao, V., Shynu, R., Mehra, P., Viegas, B.E., 2010. The Nature and Distribution of Particulate Matter in the Mandovi Estuary, Central West Coast of India. Estuaries and Coasts 33, 30–44. doi:10.1007/s12237-009-9226-0

Kim, D.-J., Lee, D.-I., Keller, J., 2006. Effect of temperature and free ammonia on nitrification and nitrite accumulation in landfill leachate and analysis of its nitrifying bacterial community by FISH. Bioresour. Technol. 97, 459–468. doi:10.1016/j.biortech.2005.03.032

Klein, S., Worch, E., Knepper, T.P., 2015. Occurrence and Spatial Distribution of Microplastics in River Shore Sediments of the Rhine-Main Area in Germany. Environ. Sci. Technol. 49, 6070–6076. doi:10.1021/acs.est.5b00492

Lambert, S., Sinclair, C., Boxall, A., 2014. Occurence, Degradation and Effect of Polymer-Based Materials in the Environment. Rev. Environ. Contam. Toxicol.

227, 1–53. doi:10.1007/978-3-319-01327-5_1

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., Schludermann, E., 2014. The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ. Pollut. 188, 177–181. doi:10.1016/j.envpol.2014.02.006

Lee, H., Shim, W.J., Kwon, J.-H., 2014. Sorption capacity of plastic debris for hydrophobic organic chemicals. Sci. Total Environ. 470–471, 1545–1552. doi:10.1016/j.scitotenv.2013.08.023

Lithner, D., Damberg, J., Dave, G., Larsson, Å., 2009. Leachates from plastic consumer products – Screening for toxicity with *Daphnia magna*. Chemosphere 74, 1195–1200. doi:10.1016/j.chemosphere.2008.11.022

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (*Danio rerio*) and Toxic Effects in Liver. Environ. Sci. Technol. 50, 4054–4060. doi:10.1021/acs.est.6b00183

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., Nava-Saucedo, J.-E., 2008. Polymer biodegradation: Mechanisms and estimation techniques – A review. Chemosphere 73, 429–442. doi:10.1016/j.chemosphere.2008.06.064

Ludke, J.L., Hill, E.F., Dieter, M.P., 1975. Cholinesterase (ChE) response and related mortality among birds fed ChE inhibitors. Arch. Environ. Contam. Toxicol. 3, 1–21.

Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99. doi:10.1016/j.marpolbul.2012.11.028

Maltby, L., 1994. Stress, shredders and streams: using gammarus energetics to assess water quality. In SutcliffeDW, ed, Water quality & stress indicators in marine and freshwater systems: linking levels of organisation. Freshw. Biol. Assoc. 3, 98–110.

Mani, T., Hauk, A., Walter, U., Burkhardt-Holm, P., 2015. Microplastics profile along the Rhine River. Sci. Rep. 5, 17988. doi:10.1038/srep17988

Martins, J., Sobral, P., 2011. Plastic marine debris on the Portuguese coastline: A

matter of size? Mar. Pollut. Bull. 62, 2649–2653. doi:10.1016/j.marpolbul.2011.09.028

Martins, J.V.F., 2011. Caracterização de resíduos plásticos na Costa Portuguesa – será um microproblema? New University of Lisbon, Lisbon, Portugal.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. Environ. Sci. Technol. 35, 318–324. doi:10.1021/es0010498

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River. Environ. Sci. Technol. 48, 11863–11871. doi:10.1021/es503610r

McGoran, A.R., Clark, P.F., Morritt, D., 2017. Presence of microplastic in the digestive tracts of European flounder, *Platichthys flesus*, and European smelt, *Osmerus eperlanus*, from the River Thames. Environ. Pollut. 220, 744–751. doi:10.1016/j.envpol.2016.09.078

Mintenig, S., 2014. Planktonic Microplastic in the North Sea - A new extraction method for the detection by Fourier Transform Infrared Spectroscopy (FTIR). Master Thesis. Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany.

Moatar, F., Person, G., Meybeck, M., Coynel, A., Etcheber, H., Crouzet, P., 2006. The influence of contrasting suspended particulate matter transport regimes on the bias and precision of flux estimates. Sci. Total Environ. 370, 515–531. doi:10.1016/j.scitotenv.2006.07.029

Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ. Res. 108, 131–139. doi:10.1016/j.envres.2008.07.025

Morasch, B., Bonvin, F., Reiser, H., Grandjean, D., de Alencastro, L.F., Perazzolo, C., Chèvre, N., Kohn, T., 2010. Occurrence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part II: Micropollutant removal between wastewater and raw drinking water. Environ. Toxicol. Chem. 29, 1658–1668. doi:10.1002/etc.222

MSFD GES Technical Subgroup on Marine Litter, 2013. Monitoring Guidance for Marine Litter in European Seas, Draft Report. European Commission, Brussels.

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. Environ. Sci. Technol. 50, 5800–5808. doi:10.1021/acs.est.5b05416

Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217. doi:10.1016/j.marpolbul.2011.03.032

Newman, S., Watkins, E., Farmer, A., Brink, P. ten, Schweitzer, J.-P., 2015. The Economics of Marine Litter, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 367–394. doi:10.1007/978-3-319-16510-3_14

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environ. Sci. Process. Impacts 18, 1050–1059. doi:10.1039/C6EM00206D

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J.W., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2047–2062. doi:10.1098/rstb.2008.0242

Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indic. 34, 641–647. doi:10.1016/j.ecolind.2013.06.019

PlasticsEurope, 2016. Plastics - the Facts 2016: An analysis of European plastics production, demand and waste data 1–37.

Polis, G.A., Anderson, W.B., Anderson, R., 1997. Toward an Integration of Landscape and Food Web Ecology : The Dynamics of Spatially Subsidized Food Webs. Annu. Rev. Ecol. Syst. 28, 289–316.

Rech, S., Macaya-Caquilpán, V., Pantoja, J.F., Rivadeneira, M.M., Jofre Madariaga, D., Thiel, M., 2014. Rivers as a source of marine litter – A study from the SE Pacific. Mar. Pollut. Bull. 82, 66–75. doi:10.1016/j.marpolbul.2014.03.019

Reisser, J., Shaw, J., Hallegraeff, G., Proietti, M., Barnes, D.K.A., Thums, M., Wilcox, C., Hardesty, B.D., Pattiaratchi, C., 2014. Millimeter-Sized Marine

Plastics: A New Pelagic Habitat for Microorganisms and Invertebrates. PLoS One 9, e100289. doi:10.1371/journal.pone.0100289

Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends Anal. Chem. 65, 47–53. doi:10.1016/j.trac.2014.10.011

Rochman, C.M., Browne, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H.K., Rios-Mendoza, L.M., Takada, H., Teh, S., Thompson, R.C., 2013a. Policy: Classify plastic waste as hazardous. Nature 494, 169–71. doi:10.1038/494169a

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013b. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263. doi:10.1038/srep03263

Rosenkranz, P., Chaudhry, Q., Stone, V., Fernandes, T.F., 2009. A comparison of nanoparticle and fine particle uptake by *Daphnia magna*. Environ. Toxicol. Chem. 28, 2142–2149. doi:10.1897/08-559.1

Ryan, P.G., Moore, C.J., van Franeker, J. a, Moloney, C.L., 2009. Monitoring the abundance of plastic debris in the marine environment. Philos. Trans. R. Soc. B Biol. Sci. 364, 1999–2012. doi:10.1098/rstb.2008.0207

Sá, S., Bastos-Santos, J., Araújo, H., Ferreira, M., Duro, V., Alves, F., Panta-Ferreira, B., Nicolau, L., Eira, C., Vingada, J., 2016. Spatial distribution of floating marine debris in offshore continental Portuguese waters. Mar. Pollut. Bull. 104, 269–278. doi:10.1016/j.marpolbul.2016.01.011

Sanchez, W., Bender, C., Porcher, J.-M., 2014. Wild gudgeons (*Gobio gobio*) from French rivers are contaminated by microplastics: Preliminary study and first evidence. Environ. Res. 128, 98–100. doi:10.1016/j.envres.2013.11.004

Schindler, D.E., Scheuerell, M.D., 2002. Habitat coupling in lake ecosystems. Oikos 98, 177–189. doi:10.1034/j.1600-0706.2002.980201.x

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83. doi:10.1016/j.envpol.2013.10.013

Shaw, D.G., Day, R.H., 1994. Colour- and form-dependent loss of plastic micro-

debris from the North Pacific Ocean. Mar. Pollut. Bull. 28, 39–43. doi:10.1016/0025-326X(94)90184-8

Simpson, B.S.L., Batley, G.E., Chariton, A.A., Stauber, J.L., King, C.K., Chapman, J.C., Hyne, R. V, Gale, S.A., Roach, A.C., Maher, W.A., Simpson, S.L., 2005. Handbook for Sediment Quality Assessment Quality Assessment. CSIRO, Bangor, NSW.

Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in Taihu Lake, China. Environ. Pollut. 216, 711–719. doi:10.1016/j.envpol.2016.06.036

Talsness, C.E., Andrade, A.J.M., Kuriyama, S.N., Taylor, J. a, vom Saal, F.S., 2009. Components of plastic: experimental studies in animals and relevance for human health. Philos. Trans. R. Soc. B Biol. Sci. 364, 2079–2096. doi:10.1098/rstb.2008.0281

Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M., Watanuki, Y., 2013. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Mar. Pollut. Bull. 69, 219–222. doi:10.1016/j.marpolbul.2012.12.010

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for Plastics to Transport Hydrophobic Contaminants. Environ. Sci. Technol. 41, 7759–7764. doi:10.1021/es071737s

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Bjorn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. doi:10.1098/rstb.2008.0284

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838. doi:10.1126/science.1094559

Thompson, R.C., Swan, S.H., Moore, C.J., vom Saal, F.S., 2009. Our plastic age.

Philos. Trans. R. Soc. B Biol. Sci. 364, 1973–1976. doi:10.1098/rstb.2009.0054

Urgert, W., 2015. Microplastics in the rivers Meuse and Rhine. Master thesis. Open University of the Netherlands, Heerlen, Netherlands.

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015. Microplastics in sediments: A review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17. doi:10.1016/j.marenvres.2015.06.007

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. doi:10.1016/j.envpol.2014.06.010

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuar. Coast. Shelf Sci. 130, 54–61. doi:10.1016/j.ecss.2013.03.022

von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel *Mytilus edulis* L. after an Experimental Exposure. Environ. Sci. Technol. 46, 11327–11335. doi:10.1021/es302332w

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 12. doi:10.1186/s12302-014-0012-7

Wang, J., Peng, J., Tan, Z., Gao, Y., Zhan, Z., Chen, Q., Cai, L., 2017. Microplastics in the surface sediments from the Beijiang River littoral zone: Composition, abundance, surface textures and interaction with heavy metals. Chemosphere 171, 248–258. doi:10.1016/j.chemosphere.2016.12.074

Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033. doi:10.1016/j.cub.2013.10.068

Zbyszewski, M., Corcoran, P.L., Hockin, A., 2014. Comparison of the distribution and degradation of plastic debris along shorelines of the Great Lakes, North America. J. Great Lakes Res. 40, 288–299. doi:10.1016/j.jglr.2014.02.012

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “Plastisphere”: Microbial Communities on Plastic Marine Debris. Environ. Sci. Technol. 47, 7137–7146. doi:10.1021/es401288x

# Chapter 2

# Microplastics in freshwater systems: effectiveness of distinct methods for the separation of microplastics

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## Chapter 2 – Microplastics in freshwater systems: effectiveness of distinct methods for the separation of microplastics


### Abstract

The accumulation of plastics in aquatic systems constitutes an emerging scientific and societal concern, because of their ubiquity, high persistence and insufficient management by sewage treatment plant and wastewater treatment. Microplastics (< 5 mm), a group of particles differing in physico-chemical properties (e.g. size, shape, colour, density and polymer type), are of particular apprehension as they can reach high densities and can interact with biotic and abiotic environment. Moreover, potential of bioaccumulation increases with decreasing of particles' size. Although microplastics have been widely investigated in marine systems, very little attention is paid to freshwater systems. As the concern about microplastics started appearing recently, there is no unified method for microplastic separation, which result in inaccuracy data that differs in quality and resolution. Hence, this work aims to assess the effectiveness of distinct separation methods as an attempt to identify and establish a unified method. For that, artificial samples containing eleven plastics belonging to the most common types of polymers (e.g. low/high-density polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate) were prepared and subjected to different methods, including density separation methods using sugar, olive oil and zinc chloride, as well as organic matter degradation methods with hydrogen peroxide (wet peroxide oxidation) and multienzymatic detergent (enzymatic digestion). The samples were then undergoing the detection, quantification and identification of polymers using a stereomicroscope and Fourier transform infrared spectroscopy (FTIR). Several criteria were considered in order to achieve the aims of this work: efficiency of density separation and organic matter degradation, the total mass of recovered polymers, cost of each procedure, the time spent with each method, the simplicity and the quality of recovered polymers. Based on this multi-criteria approach, this study concludes that the wet peroxide oxidation with addition of zinc chloride was the most cost-effective method.

**Keywords**
Microplastics; freshwater systems; separation methods.

## 1. Introduction

Since the middle of the 20th century, the worldwide production of plastics has increased exponentially reaching 322 million tonnes of plastics in 2015 (PlasticsEurope, 2016). Plastics possess a unique set of properties such as lightness, inexpensive, versatility, durability, resistance and strength (Thompson et al., 2009) that provide remarkable benefits (e.g. technological advances and energy savings) for many industries and almost every sector of our everyday life (Andrady, 2011; Andrady and Neal, 2009; Dris et al., 2015). According to PlasticsEurope (2016), the preference for more ecological management options regarding plastics waste are increasing (e.g. recycling and energy recovery). Notwithstanding, in many EU countries landfill stills the first option (30.8%). The insufficient waste management coupled with high production, physical characteristics (chemical inertness and slow biodegradation) and improper waste disposal (e.g. industry, urban waste, sewage treatment plant – STP, agriculture, accidental) results in an accumulation of plastic debris in the environment, in particular in aquatic systems (Barnes et al., 2009; Dris et al., 2015; Eubeler et al., 2010; Thompson et al., 2004; Urgert, 2015). This contamination not only includes plastic debris with large size (macroplastics) but also microplastics (Dris et al., 2015). Currently, microplastics (MPs), usually known as particles with less than 5 mm (Arthur et al., 2009), can differ in shape, colour, specific density and polymer type as well as in their origin (primary if they are produced in a micro-size range for direct use or as precursors to other products; or secondary if they result from the continuous fragmentation of macroplastics caused by a combination of abiotic and biotic mechanisms) (Barnes et al., 2009; Cole et al., 2011; Duis and Coors, 2016; Eubeler et al., 2010; Lucas et al., 2008; Mintenig, 2014).

MPs are considered contaminants of emerging scientific and societal concern (Wagner et al., 2014), as they can reach high densities (e.g. 6 698,264 particles km-

$^{2}$, according to McCormick et al. (2014)) and can interact with abiotic (e.g. Arthur and Baker, 2011; Simpson et al., 2005) and biotic environment (e.g. Blarer and Burkhardt-Holm, 2016; de Sá et al., 2015; Green et al., 2017; Huerta Lwanga et al., 2016; Neves et al., 2015; Oliveira et al., 2013; Rehse et al., 2016; Rochman et al., 2013; Silva-Cavalcanti et al., 2017; Tosetto et al., 2017). This interaction causes negative impacts in organisms such as physical impacts (e.g. blocked digestive tracts, debilitation, limited predator avoidance, early tumour formation or death/immobilisation) (de Sá et al., 2015; Eerkes-Medrano et al., 2015) and/or toxic impacts (e.g. liver stress response or inhibition of acetylcholinesterase activity) (Oliveira et al., 2013; Rochman et al., 2013) that can induce cascading effects with trophic and ecosystem consequences (Eerkes-Medrano et al., 2015; Lechner et al., 2014; Schindler and Scheuerell, 2002). The toxic impacts can be provoked by MPs and/or contaminants and pathogens that adsorb to them (Oliveira et al., 2013; Rochman et al., 2013). Moreover, potential for bioaccumulation increases with decreasing of particles' size.

Although micro-debris is not a new problem, only recently data on MPs pollution increased rapidly (Faure et al., 2012; GESAMP, 2015). The studies about MPs in aquatic ecosystems (between 2004-2017) are mainly focused on marine systems (85.03%), while freshwater systems have received very little attention (14.97%). In addition, for several authors rivers are being seen as an important carriage systems of MPs from terrestrial to marine environment (Eerkes-Medrano et al., 2015; Hidalgo-ruz et al., 2012; Klein et al., 2015; Wagner et al., 2014), acting as temporary sinks (Blair et al., 2017).

As the concern about MPs started appearing recently, there is no unified methods for MPs detection and monitoring (sample collection and preparation; MPs identification and quantification) in freshwater systems. This could result in inaccuracy data that differs in quality and resolution, not allowing data comparison between different studies (large-scale spatial and temporal comparisons) (Duis and Coors, 2016; Löder and Gerdts, 2015). The development of a simple, low-cost and accurate method, as well as one that minimize contamination is a main challenge of the scientific community (Eerkes-Medrano et al., 2015). Sample analysis is one of the most questionable procedure that commonly consists in size fraction sieving,

organic matter removal, density separation, filtration, visual sorting and Fourier transform infrared spectroscopy (FTIR) identification (Cole et al., 2014; Hidalgo-ruz et al., 2012; Masura et al., 2015; Qiu et al., 2016; Tagg et al., 2015). Similar approaches to those implemented in marine environments have been used for freshwater systems (Blair et al., 2017; Hidalgo-ruz et al., 2012).

Hence, this work aims to identify and establish a cost-effective method, based on a multi-criteria approach, including: cost; density separation and organic matter degradation efficiency; total mass of recovered polymers; time spent with each method; and simplicity and quality of recovered polymers. For that, artificial freshwater samples containing eleven plastic products were prepared and subjected to seven distinct methods, selected according to their common application and efficiency: density separation methods using sugar, olive oil and zinc chloride, as well as organic matter degradation methods using hydrogen peroxide (wet peroxide oxidation) and multienzymatic detergent (enzymatic digestion).

## 2. Materials and Methods

### *2.1 Microplastic preparation*

Eleven different plastic products widely used in everyday life were broken down by various physical methods (e.g. scissors and coffee grinder) into secondary MPs (< 5 mm). Based on package label (e.g. Resin Identification Code (RIC) – recycling code (ASTM, 2013)), literature (Driedger et al., 2015; Kim et al., 2006; Mintenig, 2014; Wagner et al., 2014) and visual inspection each MPs sample was characterized by its colour, shape, reference density and, in some cases, polymer type (*see* Table 1). Although of varying shape, these secondary MPs were mostly fragments. The samples were undergoing FTIR to confirm the previous identification (*see* Appendix A). These 11 plastics products contained 5 of the most common types of polymers (PlasticsEurope, 2016) such as low/high-density polyethylene (LD/HDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET).

**Table 1 –** Identification and characterization of the 11 secondary MPs, according to the information obtained from package label/FTIR, visual inspection and literature.

| Polymer type | RIC | Source | Colour | Shape | Density (g $cm^{-3}$) |
|---|---|---|---|---|---|
| Low-density polyethylene (LDPE) | 4 | Supermarket bag | White<br>Red | Film | 0.89 – 0.93 |
| Polyethylene (PE) | | Milk box (Tetra Pak® packaging) | Blue<br>Beige<br>Silver | Fragment<br>Fiber | 0.89 – 0.98 |
| | | Toilet paper packaging | Clear<br>Green | Fragment | |
| High-density polyethylene (HDPE) | 2 | Liquid yogurt | White | Fragment | 0.94 – 0.98 |
| Polypropylene (PP) | 5 | Pasta packaging | Clear<br>White<br>Yellow<br>Green<br>Black<br>Red<br>Orange | Film | 0.85 – 0.92 |
| | | Straws<br>Rope | Yellow<br>Clear | Fragment | |
| Polystyrene (PS) | 6 | Solid yogurt | Pink<br>Green<br>Yellow | Fragment | 1.04 – 1.09 |
| Polyvinyl chloride (PVC) | 3 | Pipe | Grey | Fragment | 1.16 – 1.58 |
| Polyethylene terephthalate (PET) | 1 | Fabric<br>Water bottles | Blue<br>Blue | Fiber<br>Fragment | 1.37 – 1.45 |

### *2.2 Samples preparation*

In order to represent a realistic freshwater system with controlled contamination (only with MPs), artificial samples containing synthetic freshwater (75 mL), different types of MPs (*see* above; 0.05 g each), organic matter (cladocerans – 25 organisms, duckweed – 6 organisms and chestnut leaves – 0.05 g) and sediment (sand – 2 g) were prepared. The artificial samples were stirred for 5/10 minutes and allowed to stand overnight. Based on Smith et al. (2002), synthetic freshwater was prepared by adding magnesium chloride hexahydratade ($MgCl_2 \cdot 6H_2O$), calcium chloride ($CaCl_2$), sodium sulfate ($Na_2SO_4$), potassium bicarbonate ($KHCO_3$) and sodium bicarbonate ($NaHCO_3$) to Milli-Q water and stirred for 30 minutes.

Following samples preparation, they were passed through two sieves (55 µm and 0.2 mm) with Milli-Q water, based on Masura et al. (2015) and Hidalgo-ruz et al. (2012). The first sieve was used to determine the mass of solids in each sample and the second one to select the MPs between 5 mm and 0.2 mm. This last fraction was rinsed with Milli-Q water and transferred to flasks, while material sized < 0.2 mm were discarded. Next, samples were dried in the oven at 90ºC and weighed. Each fraction was later used for the MPs separation after application of different methods (*see* Figure 1).

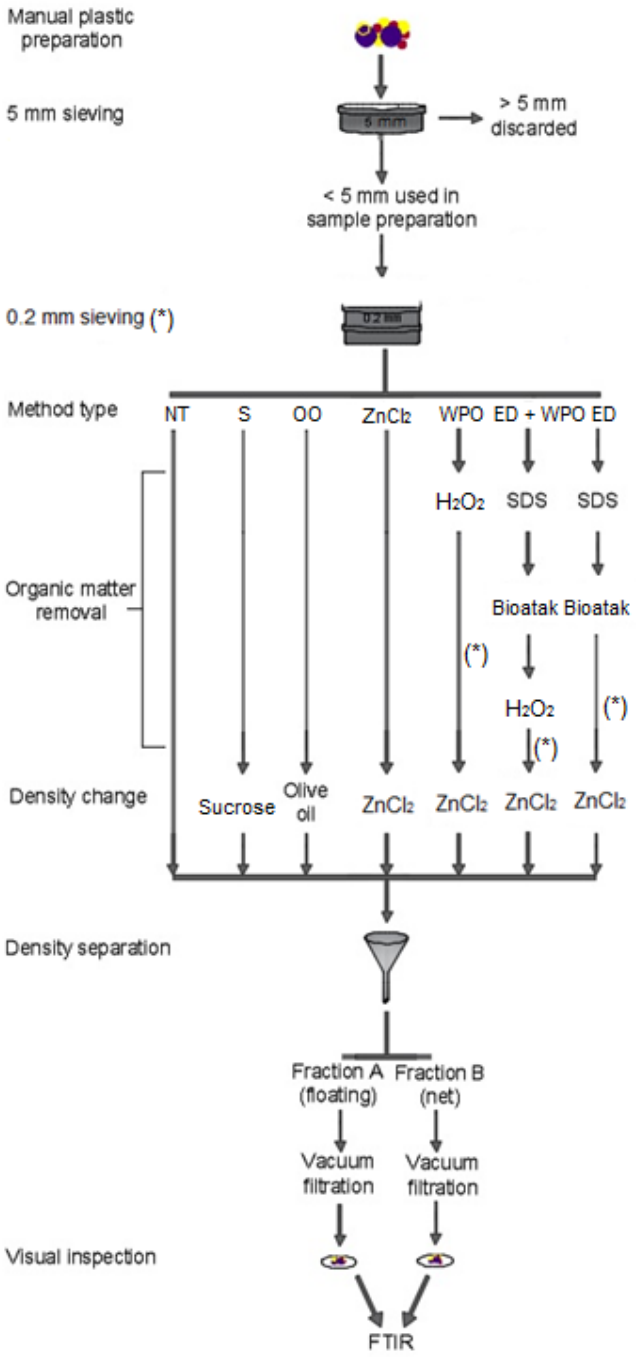


**Figure 1** – Experimental draw of all processes performed in this work including MPs and sample preparation, sample processing and FTIR identification. The star next to the arrows (*) indicate that samples were subjected again to 0.2 mm sieving.

### *2.3 Samples processing*

#### *2.3.1 Methods application*

As described in Figure 1, the fraction ≥ 0.2 mm were subjected to seven different methods for MPs separation, whose selection was based on Cedro and Cleary (2015), Hidalgo-ruz et al. (2012), Löder and Gerdts (2015), Mani et al. (2015), Masura et al. (2015), Mintenig (2014), Urgert (2015). It includes density separation methods using sugar, olive oil and zinc chloride, as well as organic matter degradation methods using an oxidizing agent (hydrogen peroxide) and a multienzymatic detergent (BIOATAK; obtained from EcoBacterias company) (see description below). A treatment where any method was applied passing directly to the density separator (no treatment – NT) was also tested. Hence, in total, the experiment consisted of 7 treatments with 5 replicas each.

After ~3/4 hours and at room temperature (Mani et al., 2015), the MPs were collected from the top into a flask (fraction A), while settled solids were drained for a 55 µm net (fraction B) and rinsed with Milli-Q water until all the particles were placed in a flask (Masura et al., 2015), for further vacuum filtration and visual inspection.

##### *2.3.1.1 Density separation methods*

To change the density of synthetic freshwater and allowed the polymers with higher density to float, three distinct methods were used: S – sugar (sucrose; 1666.7 g $L^{-1}$; density 1.1 g $cm^{-3}$) (Yalkowsky et al., 2010); OO – olive oil (10 mL; density 0.80-0.92 g $cm^{-3}$); and $ZnCl_2$ – zinc chloride (933.3 g $L^{-1}$; density 1.6 g $cm^{-3}$) (Zobkov and Esiukova, 2017). Each compound/substance were added to their correspondent method and stirred. After that, the samples were placed in the density separator.

*2.3.1.2 Organic matter degradation methods*

Two digesters, hydrogen peroxide and a multienzymatic detergent (which is composed by proteases, lipases, amylases and cellulases), were tested separately and together to degrade organic matter of animal and plant origin present in fractions of their correspondent treatment.

In the wet peroxide oxidation (WPO) method, the fraction was treated with 20 mL of iron (II) solution and 20 mL of hydrogen peroxide (30%), also called fenton's reagent, let stand for 5 minutes and then heated up to 75ºC (Masura et al., 2015). This mixture could boil violently if the temperature exceeds 75ºC. In the presence of iron (II) solution, hydrogen peroxide reacts to form the hydroxyl radicals ($^{\bullet}OH$), strong oxidants of organic substrates (Tang and Tassos, 1997; Tekin et al., 2006). The suggested reaction for Fenton's oxidation is given below (Xu et al., 2012):

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$$

$$^{\bullet}OH + C_xH_y \rightarrow H_2O + CO_2 + Q$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ + {}^{\bullet}OOH$$

$$Fe^{3+} + {}^{\bullet}OOH \rightarrow Fe^{2+} + {}^{\bullet}OO^- + H^+$$

$$Fe^{3+} + {}^{\bullet}OO^- \rightarrow Fe^{2+} + O_2 \quad ,$$

where $C_xH_y$ represents organic substrates and Q the final product obtained from substrates.

If all the visual organic matter were not fully oxidized, more 20 mL of $H_2O_2$ were added. After all organic matter was dissolved, the beaker was removed from hotplate, covered with aluminum foil and left for 15h.

The enzymatic digestion of organic matter (ED method) consisted of a first treatment with 2.5 g $L^{-1}$ of sodium dodecyl sulfate (SDS) (pH 8–8.2), then stirred for 10 minutes and incubated at 70ºC overnight (adapted from Mani et al. (2015) and Mintenig (2014)). After the samples cooled to room temperature, they were incubated with 20 mL of enzymatic solution (supernatant) for 3 days at 20–25ºC, constant oxygenation (> 0.05 mg $O_2$ $L^{-1}$) and pH 7–8. The enzymatic solution was prepared in a concentration of 100 g $L^{-1}$ and left aside for 30 minutes.

In the method ED + WPO, biological material was digested as well as oxidized (following the procedures described above).

Following methods application, the samples were sieved again (0.2 mm) and then went through a density separation using zinc chloride.

### *2.3.2 Vacuum filtration*

After density separation, the samples were filtered through a 0.45 µm clean membrane filter using a sand funnel connected to a vacuum system. The filtration setup was rinsed with Milli-Q water several times to ensure that no MPs were lost. Once filtration was complete, the membranes were carefully removed and placed in the oven to dry at 40ºC (Mintenig, 2014; Urgert, 2015) for 3-5 days. In the end, all membranes were undergoing visual inspection for further FTIR identification.

## *2.4 Microscope inspection + FTIR identification*

Firstly, all recovered particles from the fraction of each method were visual inspected on a stereomicroscope *Optika* using 1.5X magnification, where any resistant organic and inorganic matter was removed and MPs were isolated and weighed. The percentage of recovered MPs (efficiency of the method) was calculated following the equation (1).

$$\text{Efficiency (\%)} = \left(\frac{mass\ of\ MPS\ recovered\ on\ top\ of\ density\ separator}{mass\ of\ MPs\ added\ in\ sample\ preparation}\right) \times 100 \quad (1)$$

The identification of MPs was made by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) equipped with a Golden Gate single reflection unit (Speacac, Ltd, Orpington, U.K.) with a diamond crystal. The infrared radiation spectra (obtained in the OPUS program) has a resolution of 4 $cm^{-1}$ with 256 scans and a sensitivity of 1. Afterwards, the spectra were compared with spectra library and identified.

### *2.5 WPO experiment*

Aiming to discard any possibility of fibers elimination by WPO method, two different fibers (secondary MPs obtained from Tetra Pak® packaging and fabric), separately, were exposed to hydrogen peroxide at different volumes (10 mL or 20 mL). For that purpose, an experiment composed by twelve treatments was designed: 4 treatments were controls, 8 treatments were subjected to $H_2O_2$ (4 treatments to 10 mL and 4 to 20 mL) and each treatment consisted in 3 replicates. The sample preparation was similar to the one described in section 2.2, however 2 treatments of each volume and 2 controls contained only the correspondent fiber, synthetic freshwater and sediment (without organic matter). The remaining procedure followed the same order as described above: separation by size (55 µm and 0.2 mm), method application (WPO), density separation (zinc chloride), vacuum filtration and microscope inspection. The quality of polymers was guaranteed by FTIR and a WPO experiment with fibers (Tetra Pak® packaging and fabric). These procedures assured that polymers were not oxidized or degraded by any methodology (Blair et al., 2017).

### *2.6 Contamination mitigation*

To prevent MPs contamination during separation procedure lab coats and gloves were worn at all times, changing the gloves between steps. During all steps of sample treatment, glass vials were used however if plastic ware had to be utilized, it was covered with aluminum foil. Every material was cleaned and covered on top immediately after washing and after each step allowing the amount of time that a sample was exposed to air was very limited. The workplace was cleaned before and during each procedure. Since all the polymers were characterized in the beginning of the experiment, any others, especially fibers, were discarded and not accounted for the final mass recovered.

### *2.7 Statistical analysis*

One-way ANOVAs followed by Tukey multicomparision test were used to assess the significant differences between the distinct methods once normality and homoscedasticity of variance were demonstrated. Whenever normality failed, the data were transformed. The significance level of all statistical analyses was set at 0.05.

## 3. Results

To assess the effectiveness of distinct separation methods several criteria were considered: efficiency of density separation and organic matter degradation, cost of each procedure, the time spent with each method, the simplicity and the quality of recovered polymers (*see* Table 2) and the total mass of recovered polymers (from the top and the net of the density separator).

**Table 2 –** General evaluation of each method based on efficiency of density separation, procedure time (days)/simplicity, cost, organic matter (O.M.) degradation and quality of recovered polymers (FTIR and WPO experiment). NT – No treatment; S – Sugar; OO – Olive oil; $ZnCl_2$ – Zinc chloride; WPO – Wet peroxide oxidation with addition of zinc chloride; ED + WPO – Enzymatic digestion with addition of wet peroxide oxidation and zinc chloride; ED – Enzymatic digestion with addition of zinc chloride.

| Methods | Efficiency (%) | Procedure time (days)/ Simplicity | Cost (€) | O.M. degradation | Quality of polymers |
|---|---|---|---|---|---|
| NO | 61.16 | 0 / Yes | € | No | Yes |
| S | 64.67 | 1 / Yes | € | No | Yes |
| OO | 79.18 | 1 / Yes | € | No | Yes |
| $ZnCl_2$ | 96.84 | 1 / Yes | €€ | No | Yes |
| WPO | 95.59 | 1 / Yes | €€ | Yes | Yes |

| | | | | | |
|---|---|---|---|---|---|
| ED + WPO | 94.89 | 5 / Yes | €€ | Yes | Yes |
| ED | 95.56 | 5 / Yes | €€ | No | Yes |

Figure 2 illustrates the results obtained after application of 7 distinct methods on artificial samples. The polymer's mass recovered on top of the funnel of each method (efficiency) was significantly different between the distinct methods (Table 3), where methods were separated in three groups. The methods $ZnCl_2$, WPO, ED + WPO and ED formed the group with the highest percentages of efficiency (all above 90%), succeeded by the method OO and finally by NT and S (with the lowest percentages). Moreover, within the density separation methods, $ZnCl_2$ demonstrated to be the most efficient, with 96.83% of recovery. Most of MPs (more than 50%) were recovered from top in all methods and included supermarket bag, Tetra Pak® packaging, toilet paper packaging, liquid yogurt, pasta packaging, straws and rope. However, only after $ZnCl_2$ application, the all particles (including solid yogurt, pipe, fabric and water bottles) had the capacity to float. In general, it was possible to recover the initial mass.

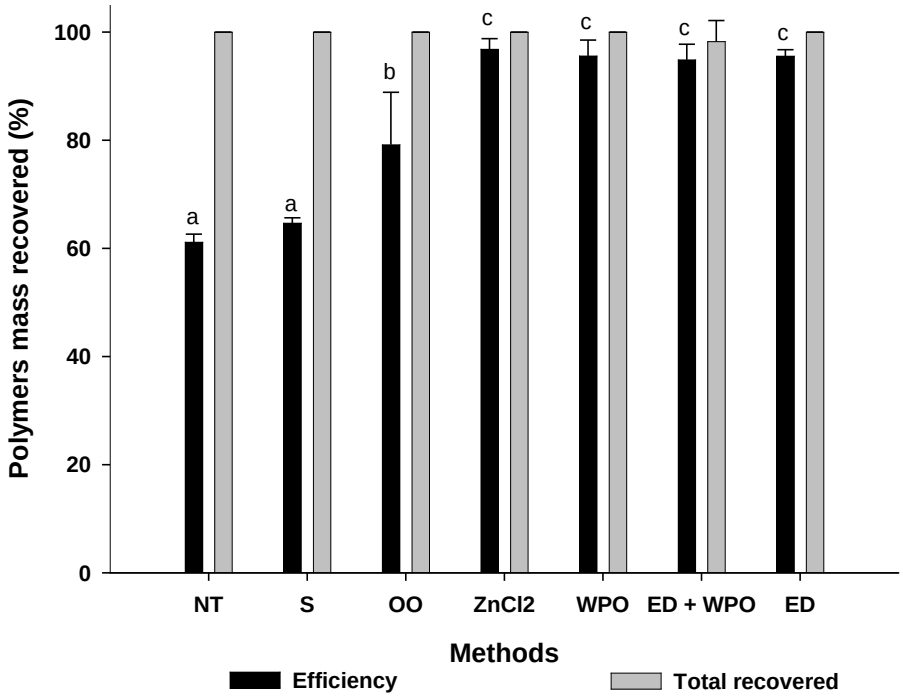


**Figure 2 –** Percentages of mass recovered from the top of funnel (efficiency) and from top and net (total recovered) after application of 7 distinct methods (NT – No treatment; S – Sugar; OO – Olive oil; $ZnCl_2$ – Zinc chloride; WPO – Wet peroxide oxidation with addition of zinc chloride; ED + WPO – Enzymatic digestion with addition of wet peroxide oxidation and zinc chloride; ED – Enzymatic digestion with addition of zinc chloride). Distinct letters over the bars indicate significant differences among methods.

**Table 3 –** One-way ANOVA summary table applied to the efficiency of the seven methods and to the WPO experiment, with particular focus on two particular polymers (fibers from fabric and Tetra Pak® packaging) after WPO exposure.

| Parameter | *DF* | *F* | *P value* |
|---|---|---|---|
| Efficiency | 30 | 101.045 | < 0.001 |
| Polyethylene terephthalate mass recovered (fiber) | 17 | 2.220 | 0.120 |
| Polyethylene's mass recovered (fiber) | 17 | 0.403 | 0.838 |

*DF* is degrees of freedom; *F* is F statistic

Regarding the methods with organic matter degradation, the WPO and ED + WPO methods, they were more effective than the enzymatic digestion isolated (ED) (Table 4). Wet peroxide oxidation caused discolouration of chestnut leaves and eliminate all cladocerans and duckweed organisms. During the extensive procedure, the multienzymatic detergent created a cloudy layer of bacteria that involve all the materials and had an intense odor. Moreover, this procedure did not degrade any organic matter and after addition of hydrogen peroxide it was created an increasing foam that could expelled MPs. ED + WPO showed the same results as WPO isolated.

**Table 4** – Semi-quantitative evaluation of the methods used for organic matter degradation, where 1 means that no organic matter was degraded (0% efficiency) and 5 that all organic matter was degraded (100% efficiency). WPO – Wet peroxide oxidation with addition of zinc chloride; ED + WPO – Enzymatic digestion with addition of wet peroxide oxidation and zinc chloride; ED – Enzymatic digestion with addition of zinc chloride.

| Methods | Degradation of organic matter efficiency (1-5) |
|---|---|
| WPO | 4 |
| ED + WPO | 4 |
| ED | 1 |

Each of tested method had a fast and a simple procedure (easy to perform), except the enzymatic digestion (ED) that took five days. Furthermore, olive oil (OO) did not disappear after filtration and dryness staying attached to MPs and increasing the time to dry the samples. Inspection of net revealed that sediment tend to attach to fibers (when density separation did not work), however when olive oil was applied, it was created a layer that increased this attachment (with fibers and other types of MPs). The cost was dependent on the number of reagents used in the procedure increasing when zinc chloride was applied. NT, S and OO were the cheapest procedures. Ultimately, the quality of recovered polymers was guaranteed for all methods based on WPO experiment and Fourier transform infrared spectroscopy's results (Figure 3). After application of two different volumes of hydrogen peroxide

(10 and 20 mL), in the presence and absence of organic matter, the recovered mass did not show significant differences (Table 3). These results revealed that WPO did not eliminate fibers, in particular PE and PET fibers. Moreover, the efficiency of elimination of organic matter was higher when 20 mL of hydrogen peroxide was applied. Based on FTIR, polymers exposed to WPO method were not degraded showing the same spectra both at the beginning and at the final of each procedure. PE and PP samples produced spectra with characteristic peaks around wavenumber regions 2915, 2849, 1471 and 717 $cm^{-1}$ and 2950, 2918, 2868, 2839, 1456 $cm^{-1}$ respectively (Figure 3).

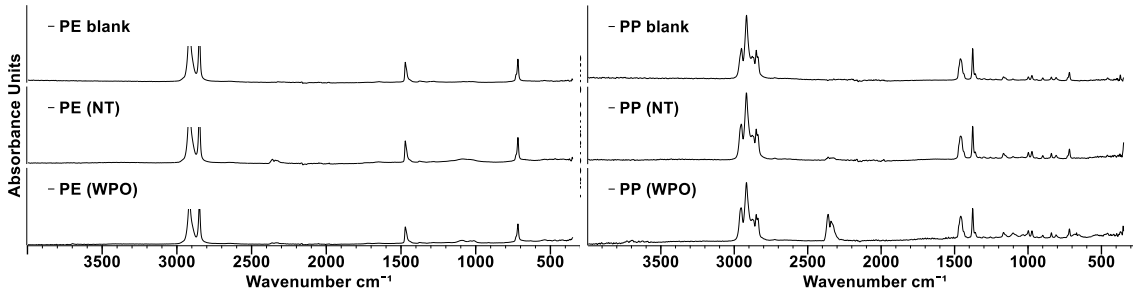


**Figure 3 –** Examples of Fourier transform infrared spectroscopy (FTIR) results obtained for polyethylene (PE) and polypropylene (PP) blanks and after exposure to NT and WPO methods.

## 4. Discussion

The concern about MPs and its impacts in aquatic ecosystems started appearing recently, however, the studies are main focused on marine systems, while freshwater systems have received very little attention (Blair et al., 2017; Duis and Coors, 2016; Eerkes-Medrano et al., 2015; Horton et al., 2017). Since freshwater systems are being seen as carriage systems of MPs from terrestrial to marine environment (Blair et al., 2017; Eerkes-Medrano et al., 2015; Hidalgo-ruz et al., 2012; Klein et al., 2015; Wagner et al., 2014), it is important its monitoring. Currently, there is no unified methods for analysis of MPs in water samples (Blair et al., 2017; Duis and Coors, 2016; Löder and Gerdts, 2015). The present work allows for a better comprehension of the advantages and disadvantages of 7 different methods allowing to infer which is the most cost-effective method. For that, several

criteria were considered like efficiency of density separation and organic matter degradation, the total mass of recovered polymers (from the top and the net of density separator), cost of each procedure, the time spent with each method, the simplicity and the quality of recovered polymers.

As expected, all methods had a percentage of efficiency above 50% because the majority of MPs used (more than 50%) are made of polymers with lower density than freshwater (≈1 g $cm^{-3}$) (Andrady, 2011; Duis and Coors, 2016; Imhof et al., 2012), such as low/high-density polyethylene and polypropylene, leading them to float naturally. This is the explanation for results obtained in the no treatment (NT), sugar (S) and olive oil (OO) methods. The solubility of sugar in water at 20-25ºC is a limitation not allowing the density reached 1.6 g $cm^{-3}$ (higher polymers' density used in this work). OO method showed a higher percentage than NT and S method, however it is an overestimation of the real results. This reagent did not mix with water which consequently will stay attached to particles and change the texture of them (they become stickiness and heavier). At last, $ZnCl_2$, WPO, ED + WPO and ED were the most efficient methods, since they shared the same density separator reagent, zinc chloride. This compound, as suggested by literature (Claessens et al., 2013; Imhof et al., 2012; Liebezeit and Dubaish, 2012; Mintenig, 2014; Nuelle et al., 2014; Van Cauwenberghe et al., 2015; Zobkov and Esiukova, 2017), can reach densities around 1.5-1.6 g $cm^{-3}$ including all types of polymers used in this work, explaining the results obtained (efficiency above 95%). The same values were also observed by Zobkov and Esiukova (2017), using similar protocol for separation of MPs from sediment samples. Polymers, such as PET and PVC, represent 17% of the European plastic demand (PlasticsEurope, 2016), an important percentage of the MPs that could be found in water. In spite of having a higher efficiency both in water and sediment, zinc chloride is mostly used for the detection of MPs in sediments (Imhof et al., 2012; Mintenig, 2014; Nuelle et al., 2014; Zobkov and Esiukova, 2017). Most of the authors prefer to use sodium chloride in water samples analysis (e.g. Browne et al., 2011, 2010; Claessens et al., 2011; Mani et al., 2015; Thompson et al., 2004). This preference is based on cost and fate of polymers in aquatic environment, where plastics with lower density than freshwater are buoyant and those with a higher density are submerged (Andrady, 2011; Imhof et al., 2012).

However, this characteristic is not the only factor that can influence the particle deposition, retention and transport in freshwater. Physical factors (hydrodynamic and geographical conditions), weather (wind, rainfall, storms, floods), human activities (littering or recycling) or other particle's characteristics (size, shape and composition) can also influence (Kessarkar et al., 2010; Moatar et al., 2006; Rocha-Santos and Duarte, 2015; Simpson et al., 2005). These factors are similar to those that affect sediment transport (Nizzetto et al., 2016). In particular, physical factors such as flow velocity, river morphology, turbulence and seasonal variability of water flows have an important role in availability of particles in water. Low flows may lead to deposition of debris, whereas high velocity flood and erosion could lead to mobilization of sediment and previously settled particles, like PET or PVC (Milliman et al., 1985; Naden et al., 2016; Walling, 2009). Since, most of these factors are unpredictable at the sample collection, the use of sodium chloride will lead to underestimation of MPs quantification. Despite this important advantage, using zinc chloride has two disadvantages: it is expensive and highly toxic to aquatic biota (Mintenig, 2014). Other compounds, such as 1.6-1.8 g $cm^{-3}$ sodium iodide (NaI) or 1.4 g $cm^{-3}$ sodium polytungstate (SPT), can be applied as well. For example, NaI is less toxic than $ZnCl_2$ (Nuelle et al., 2014) however, NaI is seventy times more expensive than NaCl (Claessens et al., 2013). The solution is reuse the $ZnCl_2$ after vacuum filtration and find a reasonable disposal for eliminate this reagent. In addition, the density separation methods isolated made handling and examination of samples more difficult, since they contained organic matter mixed with MPs. This implied an extra work in order do not underestimate results. Wet digestion protocols have been commonly employed to eliminate biological materials and facilitate the separation of MPs from natural samples (Blair et al., 2017). Among the 3 methods proposed, 30% $H_2O_2$ was the best method to remove organic matter. According to Nuelle et al. (2014), this is the ideal reagent to remove about 50% of biological matter, while the other 50% also demonstrated obvious reactions (e.g. discoloured, transparent or partly dissolved and development of gas bubbles). It is better than sodium hydroxide (NaOH) and hydrochloric acid (HCl), since organic matter did not dissolve completely or became fully transparent. Tagg et al. (2015) achieved 83% of efficiency of organic matter degradation using the same reagent. These results

enhanced by the simplicity, celerity and low cost of the procedure have made WPO one of the most widely used methods for organic matter removal (Blair et al., 2017; Imhof et al., 2012; Liebezeit and Dubaish, 2012; Nuelle et al., 2014). In this way, the density separation was facilitated, making the procedure faster, easier and more effective. However, these reactions described above can complicate visual identification since natural particulates can no longer be distinguished from MPs, which are often naturally coloured white or transparent (Hidalgo-ruz et al., 2012; Nuelle et al., 2014). Consequently, the oxidation step is only recommended if there is a large amount of organic matter that makes visual selection impossible, not being applied routinely (Nuelle et al., 2014). Literature reports a potential for $H_2O_2$ to oxidized PP and PE (Ojeda et al., 2011). According to Tagg et al. (2015), after a period of 7 days of exposure, these polymers did not show any changes in their chemical structure. The same was observed in this work, highlighting the inexistence of oxidation after at least a period of 24h of exposure. Enzymatic digestion has been recommended instead of WPO (Löder and Gerdts, 2015) and proteinase-K enzyme exhibited high performance in degradation of organic matter (97%) (Cole et al., 2014). Its efficiency is dependent of sample composition and reaction conditions (e.g. reagent concentrations, temperature and digestion time). This could be the reason for what happened in this work, the multienzymatic detergent did not degrade any organic matter. This method needs more investigation and optimization. Mintenig (2014) and Mani et al. (2015) tried also a combination of both methods (ED + WPO) and obtained good results. Since the ED isolated is not efficient, a purification with $H_2O_2$ is essential but implies more costs and a longer procedure. Moreover, these authors used one detergent and enzymes isolated, which may result better with their type of organic matter.

Overall, considering all the results, the most cost-effective method tested was wet peroxide oxidation with addition of zinc chloride. Notwithstanding its efficiency, the use of hydrogen peroxide must be cautious and only applied when absolutely necessary.

## 5. Conclusion

Important advances have been made with respect to standardization and harmonisation of separation of MPs' methodologies with this work. The majority of separation techniques are based on the same principle, however the way to achieve vary from author to author. In this study, the method more expensive (WPO + $ZnCl_2$) showed more sensitivity and, consequently, better and accuracy data. Since, MPs represent contaminants of emerging scientific and societal concern (Wagner et al., 2014) it is important its monitoring, in particular in aquatics environments. The research and regulatory community should adopt a unified method which not differ according to work aims. Moreover, it is also important the adoption of a lower size limit (Van Cauwenberghe et al., 2015), in order not to underestimate concentrations and to allow comparison between different works. This urgent situation needs to be clarified to enhance MPs research and monitoring across the world.

## 6. References


Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. doi:10.1016/j.marpolbul.2011.05.030

Andrady, A.L., Neal, M. a, 2009. Applications and societal benefits of plastics. Philos. Trans. R. Soc. B Biol. Sci. 364, 1977–1984. doi:10.1098/rstb.2008.0304

Arthur, C., Baker, J., 2011. Proceedings of the Second Research Workshop on Microplastic Marine Debris. November 5-6, 2010. NOAA Technical Memorandum NOS-OR&R-39.

Arthur, C., Baker, J., Bamford, H., 2009. Proceedings of the International Research Workshop on the Occurrence , Effects , and Fate of Microplastic Marine Debris. Sept 9-11, 2008. NOAA Technical Memorandum NOS-OR&R-30.

ASTM, 2013. ASTM D7611 / D7611M - 13e1, Standard Practice for Coding Plastic Manufactured Articles for Resin Identification. ASTM International, West Conshohocken, PA. doi:10.1520/D7611_D7611M-13E01

Barnes, D.K. a, Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B

Biol. Sci. 364, 1985–1998. doi:10.1098/rstb.2008.0205

Blair, R.M., Waldron, S., Phoenix, V., Gauchotte-Lindsay, C., 2017. Micro- and Nanoplastic Pollution of Freshwater and Wastewater Treatment Systems. Springer Sci. Rev. doi:10.1007/s40362-017-0044-7

Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. Environ. Sci. Pollut. Res. 23, 23522–23532. doi:10.1007/s11356-016-7584-2

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. Environ. Sci. Technol. 45, 9175–9179. doi:10.1021/es201811s

Browne, M.A., Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris along estuarine shorelines. Environ. Sci. Technol. 44, 3404–3409. doi:10.1021/es903784e

Cedro, A., Cleary, J., 2015. Microplastics in Irish Freshwaters : a Preliminary Study, in: Proceedings of the 14th International Conference on Environmental Science and Technology. Rhodes, Greece, pp. 1–4.

Claessens, M., Meester, S. De, Landuyt, L. Van, Clerck, K. De, Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62, 2199–2204. doi:10.1016/j.marpolbul.2011.06.030

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. 70, 227–233. doi:10.1016/j.marpolbul.2013.03.009

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull. 62, 2588–2597. doi:10.1016/j.marpolbul.2011.09.025

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 4528. doi:10.1038/srep04528

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles

of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. doi:10.1016/j.envpol.2014.10.026

Driedger, A.G.J., Dürr, H.H., Mitchell, K., Van Cappellen, P., 2015. Plastic debris in the Laurentian Great Lakes: A review. J. Great Lakes Res. 41, 9–19. doi:10.1016/j.jglr.2014.12.020

Dris, R., Imhof, H., Sanchez, W., Gasperi, J., Galgani, F., Tassin, B., Laforsch, C., 2015. Beyond the ocean: contamination of freshwater ecosystems with (micro-)plastic particles. Environ. Chem. 12, 539. doi:10.1071/EN14172

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28, 2. doi:10.1186/s12302-015-0069-y

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82. doi:10.1016/j.watres.2015.02.012

Eubeler, J.P., Bernhard, M., Knepper, T.P., 2010. Environmental biodegradation of synthetic polymers II. Biodegradation of different polymer groups. TrAC Trends Anal. Chem. 29, 84–100. doi:10.1016/j.trac.2009.09.005

Faure, F., Corbaz, M., Baecher, H., de Alencastro, L.F., 2012. Pollution due to plastics and microplastics in Lake Geneva and in the Mediterranean Sea. Arch. Sci. 65, 157–164.

GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment, Kershaw, P.J., ed. Rep. Stud. GESAMP No. 90, 96 p. doi:10.13140/RG.2.1.3803.7925

Green, D.S., Boots, B., O'Connor, N.E., Thompson, R., 2017. Microplastics Affect the Ecological Functioning of an Important Biogenic Habitat. Environ. Sci. Technol. 51, 68–77. doi:10.1021/acs.est.6b04496

Hidalgo-ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environ. Sci. Technol. 46, 3060−3075.

doi:dx.doi.org/10.1021/es2031505

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141. doi:10.1016/j.scitotenv.2017.01.190

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the Terrestrial Ecosystem: Implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). Environ. Sci. Technol. 50, 2685–2691. doi:10.1021/acs.est.5b05478

Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P., Laforsch, C., 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnol. Oceanogr. Methods 10, 524–537. doi:10.4319/lom.2012.10.524

Kessarkar, P.M., Purnachandra Rao, V., Shynu, R., Mehra, P., Viegas, B.E., 2010. The Nature and Distribution of Particulate Matter in the Mandovi Estuary, Central West Coast of India. Estuaries and Coasts 33, 30–44. doi:10.1007/s12237-009-9226-0

Kim, D.-J., Lee, D.-I., Keller, J., 2006. Effect of temperature and free ammonia on nitrification and nitrite accumulation in landfill leachate and analysis of its nitrifying bacterial community by FISH. Bioresour. Technol. 97, 459–468. doi:10.1016/j.biortech.2005.03.032

Klein, S., Worch, E., Knepper, T.P., 2015. Occurrence and Spatial Distribution of Microplastics in River Shore Sediments of the Rhine-Main Area in Germany. Environ. Sci. Technol. 49, 6070–6076. doi:10.1021/acs.est.5b00492

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., Schludermann, E., 2014. The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ. Pollut. 188, 177–181. doi:10.1016/j.envpol.2014.02.006

Liebezeit, G., Dubaish, F., 2012. Microplastics in Beaches of the East Frisian Islands Spiekeroog and Kachelotplate. Bull. Environ. Contam. Toxicol. 89, 213–217. doi:10.1007/s00128-012-0642-7

Löder, M.G.J., Gerdts, G., 2015. Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal, in: Bergmann, M., Gutow, L., Klages, M. (Ed.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 201–227. doi:10.1007/978-3-319-16510-3_8

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., Nava-Saucedo, J.-E., 2008. Polymer biodegradation: Mechanisms and estimation techniques – A review. Chemosphere 73, 429–442. doi:10.1016/j.chemosphere.2008.06.064

Mani, T., Hauk, A., Walter, U., Burkhardt-Holm, P., 2015. Microplastics profile along the Rhine River. Sci. Rep. 5, 17988. doi:10.1038/srep17988

Masura, J., Baker, J., Foster, G., Arthur, C., 2015. Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments., NOAA Technical Memorandum NOS-OR&R-48.

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River. Environ. Sci. Technol. 48, 11863–11871. doi:10.1021/es503610r

Milliman, J.D., Huang-ting, S., Zuo-sheng, Y., H. Mead, R., 1985. Transport and deposition of river sediment in the Changjiang estuary and adjacent continental shelf. Cont. Shelf Res. 4, 37–45. doi:10.1016/0278-4343(85)90020-2

Mintenig, S., 2014. Planktonic Microplastic in the North Sea - A new extraction method for the detection by Fourier Transform Infrared Spectroscopy (FTIR). Master Thesis. Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany.

Moatar, F., Person, G., Meybeck, M., Coynel, A., Etcheber, H., Crouzet, P., 2006. The influence of contrasting suspended particulate matter transport regimes on the bias and precision of flux estimates. Sci. Total Environ. 370, 515–531. doi:10.1016/j.scitotenv.2006.07.029

Naden, P.S., Murphy, J.F., Old, G.H., Newman, J., Scarlett, P., Harman, M., Duerdoth, C.P., Hawczak, A., Pretty, J.L., Arnold, A., Laizé, C., Hornby, D.D., Collins, A.L., Sear, D.A., Jones, J.I., 2016. Understanding the controls on deposited fine sediment in the streams of agricultural catchments. Sci. Total

Environ. 547, 366–381. doi:10.1016/j.scitotenv.2015.12.079

Neves, D., Sobral, P., Ferreira, J.L., Pereira, T., 2015. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar. Pollut. Bull. 101, 119–126. doi:10.1016/j.marpolbul.2015.11.008

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environ. Sci. Process. Impacts 18, 1050–1059. doi:10.1039/C6EM00206D

Nuelle, M.-T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 184, 161–169. doi:10.1016/j.envpol.2013.07.027

Ojeda, T., Freitas, A., Birck, K., Dalmolin, E., Jacques, R., Bento, F., Camargo, F., 2011. Degradability of linear polyolefins under natural weathering. Polym. Degrad. Stab. 96, 703–707. doi:10.1016/j.polymdegradstab.2010.12.004

Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indic. 34, 641–647. doi:10.1016/j.ecolind.2013.06.019

PlasticsEurope, 2016. Plastics - the Facts 2016: An analysis of European plastics production, demand and waste data 1–37.

Qiu, Q., Tan, Z., Wang, J., Peng, J., Li, M., Zhan, Z., 2016. Extraction, enumeration and identification methods for monitoring microplastics in the environment. Estuar. Coast. Shelf Sci. 176, 102–109. doi:10.1016/j.ecss.2016.04.012

Rehse, S., Kloas, W., Zarfl, C., 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of *Daphnia magna*. Chemosphere 153, 91–99. doi:10.1016/j.chemosphere.2016.02.133

Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends Anal. Chem. 65, 47–53. doi:10.1016/j.trac.2014.10.011

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263.

doi:10.1038/srep03263

Schindler, D.E., Scheuerell, M.D., 2002. Habitat coupling in lake ecosystems. Oikos 98, 177–189. doi:10.1034/j.1600-0706.2002.980201.x

Silva-Cavalcanti, J.S., Silva, J.D.B., França, E.J. de, Araújo, M.C.B. de, Gusmão, F., 2017. Microplastics ingestion by a common tropical freshwater fishing resource. Environ. Pollut. 221, 218–226. doi:10.1016/j.envpol.2016.11.068

Simpson, B.S.L., Batley, G.E., Chariton, A.A., Stauber, J.L., King, C.K., Chapman, J.C., Hyne, R. V, Gale, S.A., Roach, A.C., Maher, W.A., Simpson, S.L., 2005. Handbook for Sediment Quality Assessment Quality Assessment. CSIRO, Bangor, NSW.

Smith, E.., Davison, W., Hamilton-Taylor, J., 2002. Methods for preparing synthetic freshwaters. Water Res. 36, 1286–1296. doi:10.1016/S0043-1354(01)00341-4

Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging. Anal. Chem. 87, 6032–6040. doi:10.1021/acs.analchem.5b00495

Tang, W., Tassos, S., 1997. Oxidation kinetics and mechanisms of trihalomethanes by Fenton's reagent. Water Res. 31, 1117–1125. doi:10.1016/S0043-1354(96)00348-X

Tekin, H., Bilkay, O., Ataberk, S., Balta, T., Ceribasi, I., Sanin, F., Dilek, F., Yetis, U., 2006. Use of Fenton oxidation to improve the biodegradability of a pharmaceutical wastewater. J. Hazard. Mater. 136, 258–265. doi:10.1016/j.jhazmat.2005.12.012

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838. doi:10.1126/science.1094559

Thompson, R.C., Swan, S.H., Moore, C.J., vom Saal, F.S., 2009. Our plastic age. Philos. Trans. R. Soc. B Biol. Sci. 364, 1973–1976. doi:10.1098/rstb.2009.0054

Tosetto, L., Williamson, J.E., Brown, C., 2017. Trophic transfer of microplastics does not affect fish personality. Anim. Behav. 123, 159–167. doi:10.1016/j.anbehav.2016.10.035

Urgert, W., 2015. Microplastics in the rivers Meuse and Rhine. Master thesis. Open

University of the Netherlands, Heerlen, Netherlands.

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015. Microplastics in sediments: A review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17. doi:10.1016/j.marenvres.2015.06.007

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 12. doi:10.1186/s12302-014-0012-7

Walling, D.E., 2009. The Impact of Global Change on Erosion and Sediment Transport by Rivers: Current Progress and Future Challenges 81, 30. doi:978-92-3-104135-8

Xu, H., Li, M., Wang, H., Miao, J., Zou, L., 2012. Fenton Reagent Oxidation and Decolorizing Reaction Kinetics of Reactive Red SBE. Energy Procedia 16, 58–64. doi:10.1016/j.egypro.2012.01.011

Yalkowsky, S.H., He, Y., Jain, P., 2010. Handbook of Aqueous Solubility Data, 2nd ed. Boca Raton, FL : CRC Press, New York. doi:10.1017/CBO9781107415324.004

Zobkov, M., Esiukova, E., 2017. Microplastics in Baltic bottom sediments: Quantification procedures and first results. Mar. Pollut. Bull. 114, 724–732. doi:10.1016/j.marpolbul.2016.10.060

# Chapter 3

# Spatial and temporal distribution of microplastics in water and sediments of Antuã River in Portugal

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## Chapter 3 - Spatial and temporal distribution of microplastics in water and sediments of the Antuã River in Portugal


### Abstract

The accumulation of plastics, especially, microplastics (particles with < 5 mm) is considered one of the most emerging aquatic pollutants. They are of particular apprehension since they can reach high densities and interact with biotic and abiotic environment. The occurrence of microplastics (MPs) in freshwater systems is less understood than in marine environment. Hence, the present study aims to fill this knowledge gap, especially on freshwater systems in Portugal. This study provides new insights into MPs abundances and distribution in Antuã river by applying the separation method of wet peroxide oxidation with addition of zinc chloride to water and sediment samples collected in March and October of 2016 in several stretches of the river. The abundance of MPs in water ranged from 5 – 8.3 mg $m^{-3}$ or 58 – 193 items $m^{-3}$ in March and from 5.8 – 51.7 mg $m^{-3}$ or 71 – 1265 items $m^{-3}$ in October. In sediments, the abundance ranged from 13.5 – 52.7 mg $kg^{-1}$ or 100 – 629 items $kg^{-1}$ in March and from 2.6 – 71.4 mg $kg^{-1}$ or 18 – 514 items $kg^{-1}$ in October. It shows that this river is severely influenced by MPs, in magnitude order similar or higher to that found in marine/coastal environment. Spatial and temporal distributions show different pattern according to seasonal conditions, proximity to urban areas and flow velocity. The water and sediment samples with the greatest abundances were São João da Madeira and Aguincheira, respectively. In water compartment, the highest abundance of MPs was observed in October, while in sediments an opposite pattern was observed. Analysis of plastics by Fourier transform infrared spectroscopy (FTIR) underline polyethylene (PE) and polypropylene (PP) polymers as the most common, which covered more than 50% of all polymer types identified. Furthermore, the oxidation ratio for these particles were 54:38:8% indicating that fewer particles are highly oxidized. Foams and fibers were the most abundant type in São João da Madeira, while fibers and fragments are the most abundant in Aguincheira and Estarreja in water and sediment samples, respectively. This study emphasizes the importance of rivers as potential carriage systems of MPs. Further studies should

be performed to identify point sources in order to mitigate the MP contamination in aquatic systems.

**Keywords**
Microplastics; Antuã river; contamination; water; sediments.

## 1. Introduction

Plastics, one of the most demanded industrial materials (322 million tonnes in 2015) (PlasticsEurope, 2016), constitute 54% of the global mass of anthropogenic waste (Hoellein et al., 2014). Currently, the fate of the majority of post-consumer plastics waste is recycling and energy recovery processes (69.2%), however in many EU countries landfill is still the first option (30.8%) (PlasticsEurope, 2016). Although the efforts, the accumulation of plastics in aquatic systems is increasing due to their ubiquity, high persistence, inadequate management and improper waste disposal (Barnes et al., 2009; Dris et al., 2015b; Eubeler et al., 2010; Thompson et al., 2004; Urgert, 2015). They are worldwide produced in a wide range of sizes (primary source), however microplastics (< 5 mm) are of particular concern as they can derive from a variety of sources, reach high densities within environment and interact with biotic and abiotic environment. These micro debris (MPs) differ in their physico-chemical properties (e.g. size, shape, colour, density and polymer type) as well as in their origin (primary or secondary) (Barnes et al., 2009; Cole et al., 2011; Duis and Coors, 2016; Eubeler et al., 2010; Lucas et al., 2008; Mintenig, 2014). Secondary MPs result from the continuous fragmentation of macroplastics caused by a combination of abiotic and biotic mechanisms (Beyler and Hirschler, 2001; Cole et al., 2011; Eubeler et al., 2010; Lucas et al., 2008). Abiotic mechanisms include photodegradation, mechanical abrasion, chemical, biological and thermal degradation and disintegration. The ingestion of these small particles, and further bioaccumulation, can cause hazardous consequences for survival, fitness, metabolism, growth, reproductive output and health of aquatic organisms (e.g. Au et al., 2015; Blarer and Burkhardt-Holm, 2016; de Sá et al., 2015; Galloway et al., 2017; Lu et al., 2016; Oliveira et al., 2013; Rochman et al., 2013) and for ecosystem

(Eerkes-Medrano et al., 2015; Lechner et al., 2014; Schindler and Scheuerell, 2002). Moreover, MPs can interact directly or indirectly with abiotic characteristics, such as alterations in light penetration and in sediment characteristics (Arthur and Baker, 2011; Simpson et al., 2005). All of these impacts can be caused by isolated MPs and/or contaminants and pathogens that tend to adsorb to the their surface (Oliveira et al., 2013; Rochman et al., 2013). Once released in the environment, these particles can be transported to aquatic ecosystems, in particular to freshwater systems, by direct inputs from the main sources of contamination, wind or rain events that washed the plastics from land to surface waters (Duis and Coors, 2016; Epa, 1992; Lambert et al., 2014; Ryan et al., 2009). They are accumulated in water column and/or in sediments that acts as temporary sinks, according to their specific characteristics (Blair et al., 2017; Castañeda et al., 2014; Eisma and Cadeé, 1991; Free et al., 2014; Imhof et al., 2013, 2012; Zbyszewski et al., 2014). Other factors, as flow velocity, water depth, storms, floods or anthropogenic activities might also influence their physical and temporal detection (Kessarkar et al., 2010; Moatar et al., 2006; Rocha-Santos and Duarte, 2015; Simpson et al., 2005).

Moreover, freshwater systems act as transport of MPs from terrestrial to marine ecosystems (Eerkes-Medrano et al., 2015; Hidalgo-ruz et al., 2012; Klein et al., 2015; Urgert, 2015; Wagner et al., 2014). It has been estimated that rivers transport between 70-80% of plastic to these systems (GESAMP, 2010). In addition, recently MPs have been reported in lakes and rivers (water column and/or sediments) on many continents such as Europe, North and South America, Africa and Asia (e.g. Anderson et al., 2017; Besseling et al., 2013; Biginagwa et al., 2016; Dris et al., 2015a; Eriksen et al., 2013; Faure et al., 2015; Mani et al., 2015; McCormick et al., 2014; Rech et al., 2014; Su et al., 2016; Wang et al., 2017). The MPs concentration detected are dependent on the proximity of source of contamination but the values are similar to those found in marine systems (from tens to thousands of MPs) (Horton et al., 2017). Mostly of freshwater systems are surrounded by high population density and, consequently, intensive anthropogenic activities increase the abundance of MPs in these areas. Furthermore, these systems have a high economic value associated, since freshwater systems are widely used in our daily lives (Eerkes-Medrano et al., 2015).

The contamination with MPs in these systems, specifically urban lakes and rivers, have received very little attention of scientific community (14.97%), along the years, compared to marine systems (Dris et al., 2015b; Wagner et al., 2014). However, literature about this topic is increasing, mostly since 2014. Although studies are increasing, there are not evidences of studies of detection and quantification of MPs in Portuguese freshwater systems and there are only 3 publications about Portuguese marine environment (Frias et al., 2016, 2013; Martins and Sobral, 2011). The MPs concentrations found in the Portuguese coast were 0.01 items $g^{-1}$ of sediment, 452 g $m^{-2}$ and 133.3 items $m^{-2}$ (beaches in Algarve, Alcobaça and along the coast, respectively). Since Portugal is the 12$^{th}$ country in Europe with the highest plastics demand (≈ 1 million tonnes) and 10% - 50% of plastic go to landfills (PlasticsEurope, 2016), it is important to understand the levels of MPs contamination in water samples and sediment in freshwater systems. However, adequate quantitative and qualitative assessment has been difficult due to their small size and lack of standard methods for sample collection, processing, characterisation and quantification (units). This leads to a huge lack of adequate data for reliable risk assessment and spatial and temporal comparisons between studies (Duis and Coors, 2016; Löder and Gerdts, 2015).

Hence, the aim of this study is to fill the gap evaluating the MPs contamination in Antuã River, in water and sediment samples collected in two seasons: Spring (March) and Autumn (October). This evaluation focused on: 1) the spatial and temporal distribution; 2) the abundance; 3) the transport and potential sources of plastic debris; and 4) the potential effects of abiotic factors (photo-oxidation: UV-B radiation) on the degradation of MPs.

## 2. Materials and Methods

### *2.1 Study area and sample collection*

The river chosen for the determination and quantification of MPs was the Antuã river, in Portugal. Its watershed has an area of 149 $km^2$ and stretches for about 38 km flowing into the Aveiro Lagoon. Antuã river is the third largest contributor of effluents to Aveiro Lagoon (average flow: 2 $m^3$ $s^{-1}$) (Dias et al., 1999).

Ínsua river, Pintor stream, Cercal stream and Arrifana stream are its main tributaries. High population density coupled with strong economic and business dynamism (main characteristics of this watershed) results in increased pressure on its water resources (Cerqueira and Silva, 2005). Water and sediments sampling was conducted at three different locations along the river (upstream to downstream: 1 and 4) São João da Madeira, 2 and 5) Aguincheira (Oliveira de Azeméis) and 3 and 6) Estarreja) at two distinct periods - March and October 2016 (Figure 1). The sites were selected based on their high and low population, urban and industrial density in the basin as well as the proximity to waste water treatment plants (WWTP), in particular to Salgueiro WWTP whose effluents destination is this river (Table 1). The collection was made mainly in artificial dam zones (on the left bank) which were quite common in this river with the objective of retain water for irrigation purposes (Moreno, 2000). Both seasons chosen were affected by rainy and wind events, which could influence the spatial distribution of particles and abundance (Dris et al., 2015b). A motor pump with a 55 µm mesh net was used for water sampling. The water was collected twice, one at the surface and other at the bottom for 5 minutes each, filtering around 1.2 $m^3$. The sample W6, an exception, was collected only at the surface due to the depth of the river was too small. One flask full of water from each site was taken for further mass of total solids determination. Two sediment samples from each site were collected with a Van Veen grab (depth of ~12 cm), from the shoreline of the river, resulting in one bulk with 0.012 $m^3$ and a sampling area of approximately 0.051 $m^2$. Overall, 12 samples were collected, of which 6 from water (W1 to W6) and 6 from sediment (S1 to S6). Moreover, the physico-chemical parameters (conductivity, oxygen saturation, temperature and pH) and flow velocity were measured in each site (*see* Appendix B). The flow velocity of the river ranged from 0 to 0.23 m $s^{-1}$. The waters sample were stored in glass jars and sediments in boxes covered by aluminium foil at 4ºC until sample process.

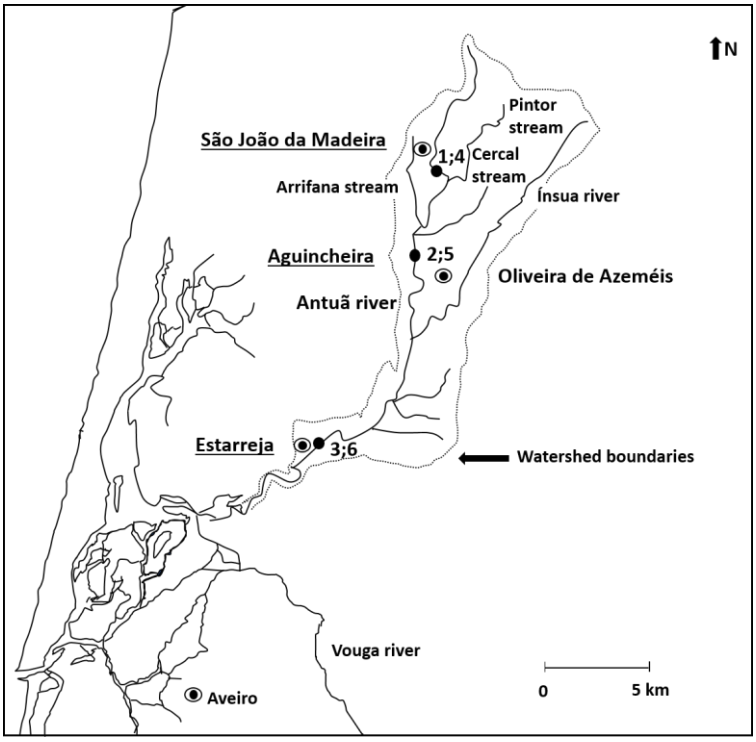


**Figure 1 –** Location of sampling sites along river Antuã are marked with black circles: (1;4) São João da Madeira, (2;5) Aguincheira and (3;6) Estarreja. Sample collection was made in two different months: March (1, 2, 3) and October (4, 5, 6).

**Table 1** – Characteristics of the river sampling locations (geographical and population data) that led to their selection for this study (INE, 2015a, 2015b).

| Location | Area ($km^2$) | Population density (inhabitants $km^{-2}$) | Reasons for selection |
|---|---|---|---|
| São João da Madeira | 7.94 | 2700.4 | High Population density<br>High Industrialization<br>High Urbanization |
| Aguincheira (Oliveira de Azeméis) | 161.1 | 415.7 | Medium Population density<br>High Industrialization<br>High Urbanization<br>Presence WWTP |
| Estarreja | 108.17 | 243.5 | Low Population density<br>High Industrialization<br>High Urbanization<br>High Tourism |

*2.2 Sample processing*

In laboratory, the analysis of the samples was performed using a modified NOAA laboratory methods (see Chapter 2; Masura et al., 2015; Zobkov and Esiukova, 2017). The water processing consisted of sieving, wet peroxide oxidation (WPO), density separation with zinc chloride and vacuum filtration. Samples were first run through a stacked series of metal sieves (5 mm and 55 µm) with Milli-Q water. Organic and plastic particles sized > 5 mm were rinsed and discarded. To analyze the samples, the fraction > 55 µm was undergone wet peroxide oxidation at approximately 75ºC for 15h to eliminate organic matter (30% $H_2O_2$ with 0.05 M Fe(II) catalyst), followed by a density separation using zinc chloride (933.3 g $L^{-1}$; density 1.6 g $cm^{-3}$). Density separation allowed MPs to float while heavier inorganic material was drained from the sample. After at least 4h, MPs were recovered and passed through a vacuum filtration (0.45 µm clean membrane filter). Once filtration was complete, the membranes were carefully transferred to the oven to dry at 40ºC for 3-5 days.

The wet sediments were first homogenized by intensive stirring with a stainless-steel spoon. After stirring a 500 g sub-sample were taken and dried at 90ºC for 2 days for determination of sediment dry weight and MPs analysis. The sediment processing had two additional steps: disaggregation of dried sediments and an extra density separation using zinc chloride. Before sieving, 400 mL of sodium polyphosphate (5.5 g $L^{-1}$) were added to the sediment sample and stirred for 1h at high rpm. Following disaggregation, the sediments were sieved (5 mm and55 µm) and it was added and stirred, with a stain-less steel spoon, 300 mL of zinc chloride separation solution (933.3 g $L^{-1}$). After 1h of settling, the supernatant was transferred to the 55 µm sieve and rinsed with Milli-Q water. The following procedure was the same of the one described above for water processing (WPO, density separation and vacuum filtration).

*2.3 Identification, quantification and oxidation of MPs*

MPs particles were visual detected under stereomicroscope *Optika* with a magnification from 1.5 x 0.8-1x. The isolated MPs were counted and weighed by classifying them according to five categories: fragments, pellets, films, foam and fibers. Several criteria were used to distinguish the particles collected (Free et al., 2014; Mani et al., 2015; Zobkov and Esiukova, 2017): 1) thick pieces with three size dimensions comparable (fragments); 2) pieces with a homogeneous sphere/spherule (pellets); 3) pieces with their thickness significantly lower than other two dimensions (films); 3) lightweight pieces (foam) and 4) thin elongated pieces with one dimension significantly greater than the other two (fibers). Particles of uncertain nature were not accounted for the final estimation. Other physical characteristics such as colour of particles were also registered and divided into four classes: white, transparent, black and colored.

An attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) equipped with a Golden Gate single reflection unit (Speacac Ltd, Orpington, UK) with a diamond crystal was used to validate MPs identification. The IR spectra were recorded with a resolution of 4 $cm^{-1}$, with 256 scans and a sensitivity of 1 (range: 4000-350 $cm^{-1}$). The analysis was made in the most frequent particles' categories choosing randomly from every sampling location (n = 43). KnowItAll Informatics Systems 7.5 software (Bio-Rada, Cambridge MA, USA) was used to match spectra of the unknown debris with reference IR-spectrums.

Oxidation was distinguished by measuring the absorbance of carbonyl and/or hydroxyl group(s), which range from 1715-1735 $cm^{-1}$ and 3200-3600 $cm^{-1}$, respectively (Reddy et al., 2008; Wang et al., 2009). The peak height of these groups was related to characteristic peak height at 1471/1450 $cm^{-1}$, compared against standard blanks. These chemical groups were not characteristic of PE, PP, PS and SBR spectra. PE characteristic spectra display peaks around 2915, 2849, 1471 and 717 $cm^{-1}$. PP also produce peaks around wave number regions 2950, 2916, 2850, 2839, 1460 and 1376 $cm^{-1}$. The main peaks of PS blanks are around 2922, 2850, 1601, 1492, 1452,753 and 695 $cm^{-1}$. SBR blanks produce the same peaks of styrene (1605, 1495, 698 $cm^{-1}$) but also butadiene wave number region

(968 $cm^{-1}$). The oxidation was expressed as low to medium to high surface oxidation ratio (%).

*2.4 Data analysis*

The total number of particles found on water was normalized to mg or items per cubic meter, while sediment samples were expressed as mg or items per kg of dry sediment. The concentration of each sample was compared using non-metric multidimensional scaling (nMDS) and the largest and the lowest contributors (plastic categories) for similarities/dissimilarities between sites were identified by a SIMPER analysis run in Primer 6 (Primer-E Ltd., Plymouth, U.K.).

*2.5 Quality assurance and quality control*

To control contamination during sample collection and processing glass vials were used and plastic material was covered with aluminum foil. After each step, material was immediately covered on the top limiting the amount of time that a sample was exposed to air and sealed with aluminum foil or parafilm. Every material and the workplace was cleaned and washed before and during each procedure. During all steps of sample analysis glass microfibers filters were placed near the samples and exposure to the same contamination. Filters were visual inspected under stereomicroscope and MPs, when appeared, counted (mainly fibers). Between 2 and 3 fibers per filter were observed, which is negligible compared to the total number of particles observed in this study.

## 3. Results

### *3.1 Abundance: spatial and temporal distribution of microplastics in water and sediment samples of Antuã river*

Depending on the unit considered, the concentration of MPs exhibits different results, hence when in doubt the results were based on items per meter cubic or kg of dry sediment (the units that showed more realistic concentrations).

Figure 2 shows the MPs concentration found in water (W1 – W6) and sediments (S1 – S6) samples along the river Antuã, expressed in milligram or items per cubic meter and milligram or items per kg of dry sediment. Micro-particles were detected in all water samples (1 – 6) with abundances ranging from 5 – 51.7 mg $m^{-3}$ or 58 – 1265 items $m^{-3}$. The most abundant weight site over all water samples was W4 (51.7 mg $m^{-3}$ or 1265 items $m^{-3}$) and the lowest abundant was W3, which was 1 or 2 orders of magnitude under those observed at other sampling sites. On average, the most abundant site was São João da Madeira whereas the lowest abundant was Estarreja. The spatial distribution of MPs in water decreased from upstream to downstream areas of the river. Seasonally, the MPs concentration found in water were higher in October 2016 (ranging from 5.8 – 51.7 mg $m^{-3}$ or 71 – 1265 items $m^{-3}$) than in March 2016 (ranging from 5 – 8.3 mg $m^{-3}$ or 58 – 193 items $m^{-3}$). Spatially, samples collected in March showed the opposite pattern of the samples collected in October, increasing the concentration along the sampling sites (1-3).

Within sediment samples, mass fraction ranged from 2.6 – 71.4 mg $kg^{-1}$ and abundance from 18 – 629 items $kg^{-1}$. Based on mass of dry sediment, the maximum MPs abundance was found in site S3 and the minimum in site S6. The most abundant site in this compartment was Aguincheira followed by Estarreja and São João da Madeira. The spatial and temporal distribution of MPs in water and sediment showed a different pattern. The MPs concentration in sediment increased upstream to downstream, with exception of Estarreja. Seasonally, the highest concentration found in sediments was in March (ranging from 13.5 – 52.7 mg $kg^{-1}$ or 100 – 629 items $kg^{-1}$) whereas in October (ranging from 2.6 – 71.4 mg $kg^{-1}$ or 18

– 514 items $kg^{-1}$) was observed the lowest concentration. Both seasons increased the abundance of MPs along the river, with the exception of S6.

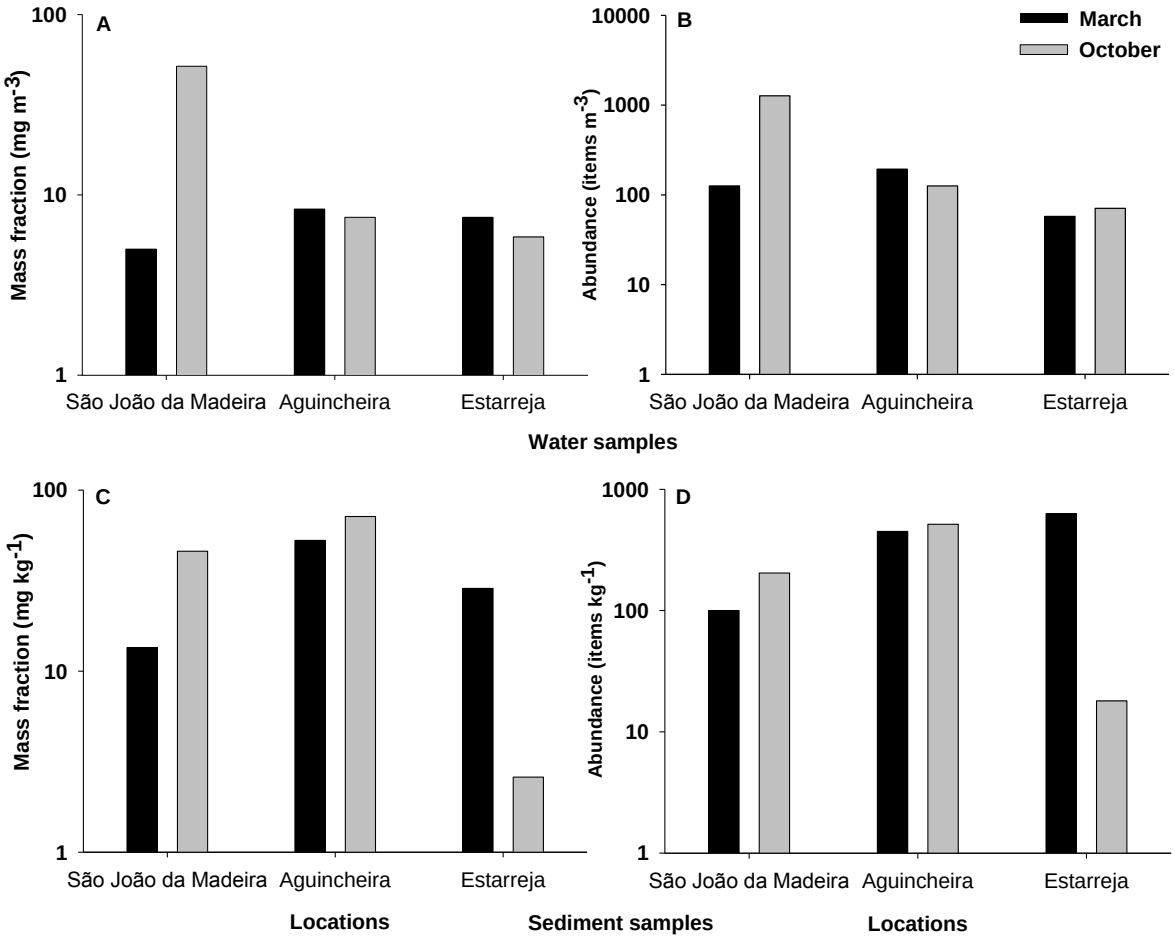


**Figure 2 –** Spatial and temporal distribution of MPs concentration along the Antuã river: A and B) Mass fraction and abundance of MPs in water samples (mg or items $m^{-3}$); C and D) Mass fraction and abundance of MPs in sediment samples (mg or items $kg^{-1}$). Data shown on logarithmic scale.

*3.2 Polymer composition and oxidation*

A total of 43 suspected particles were undergoing FTIR analysis: 34 were positively identified as synthetic polymers (79%), 3 were identified as non-plastic and 6 could not be identified (*see* Appendix C). Polyethylene (PE) and polypropylene (PP) made up > 50% of all MPs identified in water and sediments samples, followed by polystyrene (PS; mostly EPS – 8.8%), polyethylene

terephthalate (PET – 8.8%) and polyvinyl acetate (PVA – 8.8%) (Figure 3). Other polymers including ethylene-vinyl acetate (EVA), polytetrafluorethylene (PTFE), polymethylmethacrylate (PMMA), poly(ethylacrylate) (PEA), cellulose acetate and butadiene/styrene copolymer (SBR) were also identified. Although having a density lower than water, PE was commonly detected in water and sediment samples, ranging from 4-3 samples in each compartment. PP was also detected in sediment but in lower abundance compared to water samples. The other types (high and low-density polymers) were equally divided between water and sediment samples reaching a total of 8 types of polymers. The sampling sites W4 and S2 showed higher diversity of polymers compared to other locations, including some of the most common and uncommon types (e.g. PE, PP, EVA, Cellulose acetate, PEA, PVA, SBR). Seasonally, the pattern was different according to the type of sample: water samples showed higher diversity in October while sediments' samples present higher diversity in March.

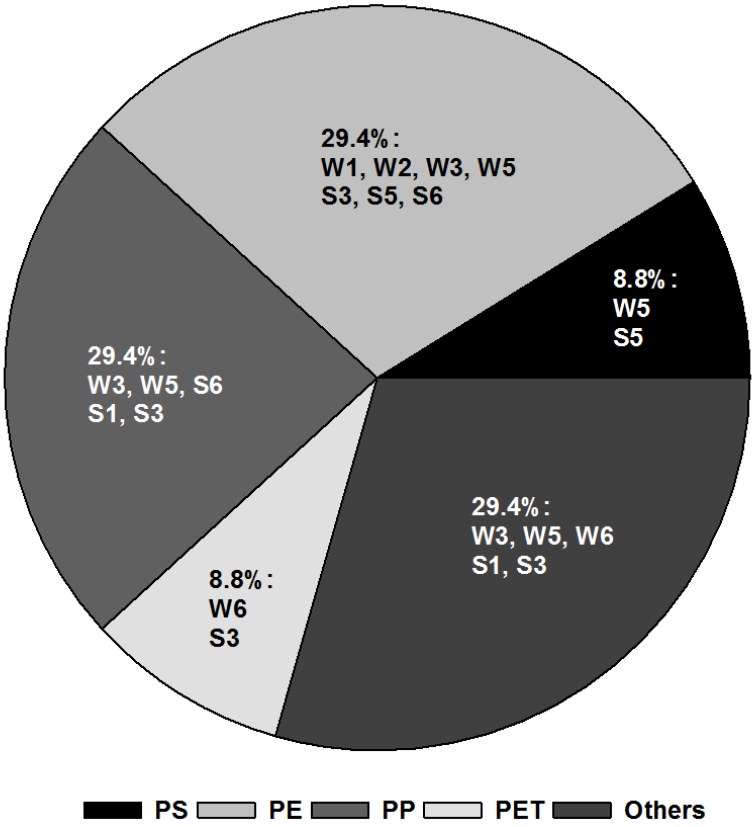


**Figure 3 –** Abundance of polymer's type identified in water and sediments of some sampling sites (n= 34).

FTIR was also used to determine the relative levels of surface oxidation in 17 selected PE, PP, PS and SBR samples from Antuã river (*see* Appendix C). This resulted in a low to medium to high surface oxidation ratio of 47:35:18 (%), based on increased peak height of carbonyl groups (e.g. carboxyl acids, aldehydes, ester and ketones) (Figure 4). PE particles were less oxidized than the other types, including 5 particles without any oxidation detected. The low to medium to high surface oxidation ratio was 54:38:8 (%) for PE and PP only.

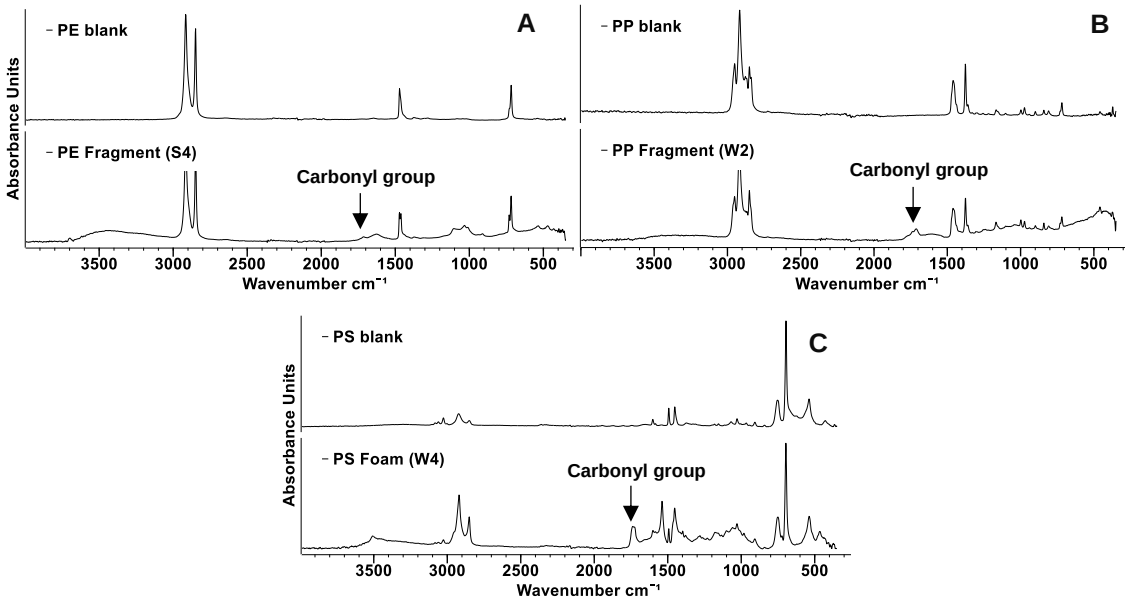


**Figure 4 –** Fourier transform infrared spectroscopy (FTIR) results from: polyethylene (PE - A), polypropylene (PP - B) and polystyrene (PS - C) blank and PE and PP fragments from site S4 (A) and W2 (B), respectively and, PS foam from site W4 (C). Note the peaks around 1712 and 1736 $cm^{-1}$ in PE fragment spectra, 1714 $cm^{-1}$ in PP and 1737 $cm^{-1}$ in PS indicates the presence of carbonyl groups formed by oxidation.

### *3.3 Microplastics' morphology*

Table 2 represents the mass fraction and numerical abundance of fragments, pellets, films, foam and fibers which differed between water and sediment samples and consequently, seasonallity. Results were expressed in items per cubic meter or kilogram of dry sediment when differences between mass and abundance were

detected. The most abundant plastic type in all water samples was foam (52.4%) and the lowest was pellets (0.05%). Furthermore, the percentage of pellets, foam and fragments found in water samples was the highest in October (100%, 99.2% and 69.6% respectively) and the lowest in March, whereas fibers and films showed an opposite trend (March: 52.4% and 51.2%, respectively). Fibers were the most dominant type of MPs found in sites W1 to W3, while in sites W4 to W6 were the foam type. The major contributor for foam dominance was the site W4. In other locations, foam was almost undetected and, pellets were only found in one sampling site (W4). Thus, foams were the most abundant type in São João da Madeira whereas fibers were the most abundant in Aguincheira and Estarreja.

Among particles that were undergoing FTIR, fragments related to sites W1-W3 were identified as PE (50%), PP (33.3%) and PTFE (16.6%). Along sites W4-W6: foam was identified as PS (50%), EVA (25%) and Cellulose Acetate (25%); films as PE (40%), PP (20%), PEA (20%) and PET (20%); fragments as PP. PS foam were identified in form of expanded PS.

In sediment, fragments (43.6%) and pellets (1.2%) were the most and lowest abundant type of MPs, respectively. The temporal distribution of plastic particles was identical to those found in water samples (October: foam – 100% and pellets - 81.8%; March: fibers – 72.1% and films – 59.7%), with exception of fragments with the highest percentage found in March (59%). The dominant type of MPs found in sites S1 to S6 was fragments, whose main contributions were from sites 2, 3, 4 and 5. Once again, foam and pellets were almost undetected, reaching only 2 samples equally divided among seasons or at the same season (respectively). Fibers were the most abundant type in São João da Madeira and fragments in Aguincheira and Estarreja. Moreover, pellets represent only a small fraction in number of particles but a larger mass fraction, in both compartments.

According to FTIR analysis, in sites S1-S3 fibers were identified as PP and PET (50% each) and fragments as PVA (42.8%), PET (14.3%), PE (14.3%), SBR (14.3%) and PP (14.3%). In addition, in sites S4-S6 pellets were identified as PEA, fragments as PMMA (20%) and PE (80%) and, foam, as PS.

Furthermore, the most abundant color group over all water and sediment samples was the colored one, followed by white, black and transparent. Fragments

were mostly blue, white and green as well as films, that changed blue through transparent. Pellets and foam were almost exclusively brown and white, respectively. Black, red, blue and transparent fibers were prevalent in almost all samples.

**Table 2 –** Shape of separated MPs along the sampling sites (W1 to W6 and S1 to S6). Results are expressed as mg or items $m^{-3}$ for water samples and mg or items $kg^{-1}$ for sediment samples.

| Season | Site | Fragments | Pellets | Films | Foam | Fibers | Total |
|---|---|---|---|---|---|---|---|
| March | W1 | 1.7/44 | 0 | 1.7/30 | 0.8/2 | 0.8/50 | 5/126 |
| | W2 | 4.2/56 | 0 | 1.7/65 | 0.8/2 | 1.7/71 | 8.4/194 |
| | W3 | 1.7/19 | 0 | 1.7/13 | 0.8/4 | 3.3/21 | 7.5/57 |
| | Total | 7.6/119 | 0 | 5.1/108 | 2.4/8 | 5.8/142 | |
| | **%** | **36.4/31.6** | **0** | **24.4/28.6** | **11.5/2.1** | **27.8/37.7** | |
| October | W4 | 8.3/213 | 1.7/1 | 14.2/68 | 25.8/947 | 1.7/36 | 51.7/1265 |
| | W5 | 2.5/48 | 0 | 1.7/26 | 1.7/9 | 1.7/43 | 7.6/126 |
| | W6 | 3.3/12 | 0 | 0.8/9 | 0 | 1.7/50 | 5.8/71 |
| | Total | 14.1/273 | 1.7/1 | 16.7/103 | 27.5/956 | 5.1/129 | |
| | **%** | **21.7/18.7** | **2.6/0.1** | **25.7/7** | **42.2/65.4** | **7.8/8.8** | |
| March | S1 | 8.1/13 | 0 | 2.7/19 | 0 | 2.7/67 | 13.5/99 |
| | S2 | 28.1/200 | 10.5/4 | 7/60 | 0 | 7/186 | 52.6/450 |
| | S3 | 10.7/279 | 0 | 7.1/154 | 0 | 10.7/194 | 28.5/627 |
| | Total | 46.9/492 | 10.5/4 | 16.8/233 | 0 | 20.4/447 | |
| | **%** | **49.6/41.8** | **11.1/0.3** | **17.8/19.8** | **0** | **21.6/38** | |
| October | S4 | 23/87 | 0 | 15.3/41 | 2.6/28 | 5.1/48 | 46/204 |
| | S5 | 17.9/255 | 17.9/18 | 17.9/116 | 4.5/18 | 13.4/107 | 71.6/514 |
| | S6 | 0 | 0 | 0 | 0 | 2.6/18 | 2.6/18 |
| | Total | 40.9/342 | 17.9/18 | 33.2/157 | 7.1/46 | 21.1/173 | |
| | **%** | **34/46.5** | **14.9/2.4** | **27.6/21.3** | **5.9/6.3** | **17.6/23.5** | |

The n-MDS analysis (Figure 5) shows the distribution of water and sediment samples based on abundance of different types of MPs found in each compartment (stress = 0). In Group A, locations of both seasons of water sampling are present with the exception of sample W4, which is in Group B. In Group C, locations of both seasons of sediment sampling are present with the exception of sample S1, which is in Group D and, sample S6, that is in Group E. The SIMPER analysis (Table 3) shows that fibers, fragments and films are the main types of MPs that contribute to the great similarity and dissimilarity within sediment groups (A; D; D/C; D/E; C/E). The dissimilarity between group A/B is also related to abundance and MPs' morphology, mainly foam and fragment type.

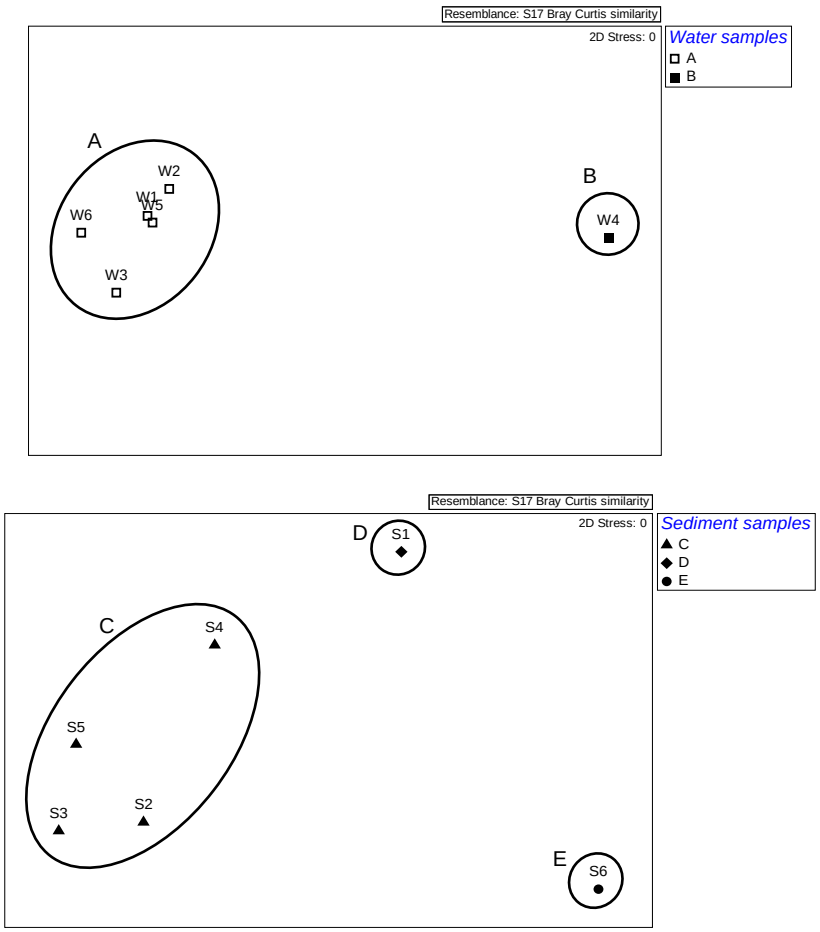


**Figure 5 –** Two-dimensional non-metric multidimensional scaling (n-MDS) ordination plot of abundance of different types of MPs of water and sediment samples from Antuã river. A (W1-W3 + W5 + W6), B (W4), C (S2-S5), D (S1) and E (S6) are the groups defined in the MDS. The results were based on abundance expressed as item $m^{-3}$ and items $kg^{-1}$.

**Table 3 –** Results of SIMPER analyses showing the types of MPs that contribute to similarity and dissimilarity within groups, according to non-metric multidimensional scaling (n-MDS) analysis.

| MDS group | Similarity | Type of MPs | Av. Abund. | Av. Sim. | Sim./SD | Contrib. (%) | Cum. (%) |
|---|---|---|---|---|---|---|---|
| A | 66.69 | Fibers | 47 | 31.99 | 3.11 | 47.96 | 47.96 |
| | | Fragments | 35.8 | 20.13 | 2.37 | 30.18 | 78.14 |
| | | Films | 28.6 | 13.35 | 2.99 | 20.02 | 98.16 |
| C | 65.57 | Fragments | 205.25 | 32.49 | 3.29 | 49.55 | 49.55 |
| | | Fibers | 134.25 | 19.16 | 2.27 | 29.23 | 78.77 |
| | | Films | 92.75 | 12.94 | 3.47 | 19.74 | 98.51 |

| MDS group | Dissimilarity | Type of MPs | Av. Abund. | | Av. Sim. | Sim./ SD | Contrib. (%) | Cum. (%) |
|---|---|---|---|---|---|---|---|---|
| A/B | 85.57 | Foam | 3.4 | 947 | 68.47 | 25.4 | 80.02 | 80.02 |
| | | Fragments | 35.8 | 213 | 12.9 | 6.9 | 15.07 | 95.09 |
| D/C | 62.91 | Fragments | 13 | 205.25 | 33.63 | 5.16 | 53.45 | 53.45 |
| | | Fibers | 67 | 134.25 | 13.05 | 1.66 | 20.74 | 74.19 |
| | | Films | 19 | 92.75 | 12.27 | 2.12 | 19.51 | 93.70 |
| D/E | 69.23 | Fibers | 67 | 18 | 41.88 | Und | 60.49 | 60.49 |
| | | Films | 19 | 0 | 16.24 | Und | 23.46 | 83.95 |
| | | Fragments | 13 | 0 | 11.11 | Und | 16.05 | 100 |
| C/E | 90.94 | Fragments | 205.25 | 0 | 43.24 | 12.04 | 47.55 | 47.55 |
| | | Fibers | 134.25 | 18 | 23.41 | 2.28 | 25.75 | 73.30 |
| | | Films | 92.75 | 0 | 19.22 | 4 | 21.14 | 94.44 |

## 4. Discussion

### *4.1 Abundance:* spatial *and temporal variations*

Our results suggested that Antuã river is polluted by MPs, especially the water compartment. Sampling sites were areas with intensive anthropogenic activities, thus it is reasonable to find high levels of contamination there (Eriksen et al., 2013). In comparison with worldwide investigations in freshwater systems, the

abundance of MPs in water was one or two orders of magnitude higher than those reported in rivers in France and Switzerland. In addition, Dris et al. (2015a) found a mean concentration of 30 and 0.35 items $m^{-3}$ in River Reine and Marne and, Faure et al. (2015) reported a mean concentration of 2.3, 0.13 and 0.29 item $m^{-3}$ in river Rhône, 0.10 item $m^{-3}$ in river Aubonne, 6.5 and 64 items $m^{-3}$ in river Venoge and 4.4 item $m^{-3}$ in river Vuachère. Both studies were performed in water collected from the surface, the lower limit was 80 µm and 300/330 µm and sodium chloride was used as density separator. Most of studies used the same procedures for sample collection and processing (e.g. Baldwin et al., 2016; Dris et al., 2015a; Faure et al., 2015; Fischer et al., 2016; Leslie et al., 2017; Mani et al., 2015; McCormick et al., 2014; Su et al., 2016). Differences in methodological approaches such as the lower limit of detection (55 µm), water sampling (i.e. collected at the surface and on the bottom) and the application of zinc chloride as density separator could be possible explanations for these distinct differences in number particles. Moreover, both sampling seasons experienced heavy precipitation and in some locations strong hydrodynamic conditions (flow velocity ranging from 0 to 0.23 m $s^{-1}$) which could lead to mobilization of sediment and previously settled particles (Ballent et al., 2016; Milliman et al., 1985; Naden et al., 2016; Walling, 2009; Zhang et al., 2017). Results of the sediments corroborate and emphasize this information, suggesting underestimation results in sediment and overestimation in water. The concentration found in river Antuã is significantly smaller than those found in river Reine, Main and Meuse (i.e. 228-3763, 786-1368 and 1400 ± 520 items $kg^{-1}$, respectively) (Klein et al., 2015; Leslie et al., 2017). Comparable amounts of plastic particles were identified in Beijiang river, where a concentration ranging from 178 to 544 items $kg^{-1}$ were separated from sediments (Wang et al., 2017). However, the concentrations of MPs found in those rivers were underestimated because was used sodium chloride as method of isolating MPs not allowing high density particles to float (e.g. PVC, PET). Sediment abundance was comparable to those reported in similar marine study (Laglbauer et al., 2014), however water concentration was significantly higher than those found in freshwater and marine studies (e.g. Baldwin et al., 2016; Carson et al., 2013; Dris et al., 2015a; Faure et al., 2015; Fischer et al., 2016; Song et al., 2014).

The spatial distribution of MPs along the river showed substantial differences between water and sediment samples. There was no significant upstream-downstream evolution of the contamination in water as expected and in sediment the pattern was not clear. This variability in abundance may be probably related to population density, flow velocity and sample collection. Locations with high density population and high flow velocity (mobilization of settled particles) showed more abundance in water, while were less abundant in the sediments. A decrease in flow facilitates the deposition of suspended particles allowing MPs to accumulate in water or sediment, according to their specific density (Milliman et al., 1985; Naden et al., 2016; Walling, 2009). Aguincheira showed always the lowest flow velocity (around 0-0.01 m $s^{-1}$) and, consequently, high levels of contamination in water and sediment. Furthermore, the water sampling in Estarreja was only made at the surface which could lead to an underestimation of the amount of MPs.

Seasonally, the abundance would be expected to be higher in water samples in March and lower in sediment, since the rain events during winter/spring were more intense than in summer/autumn. The results were not according with this assumption. The variations in water were caused by a single sample in São João da Madeira in October. The main cause could be possibly an improper waste disposal in a big scale which are directly associated with high population density and proximity to urban areas. In sediment, Estarreja was the main contributor for this variation. There is not a clear explanation for this result notwithstanding could be related to higher mobilization of sediment.

### *4.2 Polymer types, oxidation, shape and colour of microplastics particles*

The most commons polymers detected in sediment and water samples were PE and PP. These two polymers are the two most produced polymers covering 48.5% of the European plastic demand (PlasticsEurope, 2016). This allied with its specific characteristics allow to a high widespread distribution in aqueous systems (Klein et al., 2015). According to this information, these results are expected in water samples, as also described by Faure et al. (2015), Zbyszewski et al. (2014) and Zbyszewski and Corcoran (2011). Although having a density lower than freshwater,

they can submerged and be found in sediments as well (Ballent et al., 2016; Corcoran et al., 2015; Klein et al., 2015; Su et al., 2016; Vianello et al., 2013; Zhang et al., 2017). This may be related to biofouling (Andrady, 2011; McCormick et al., 2014; Zettler et al., 2013), adsorption of natural substances (Frias et al., 2016), inclusion of inorganic fillers during manufacturing (Corcoran et al., 2015) and faecal express (Cole et al., 2013; Setälä et al., 2014; Zalasiewicz et al., 2016). Furthermore, these polymers are very susceptible of photo-oxidation including physical and chemical changes such as formation of carbonyl and hydroxyl groups. Photo-oxidation, one of the most significant polymer degradation, occurs after exposure to UV-B radiation and atmospheric oxygen (McKeen, 2013). The low to medium to high surface oxidation ratio obtained for our particles is below to the ratio produced by Lake Huron (39:43:18 %) and Lake Erie (43:43:14 %) (Zbyszewski et al., 2014; Zbyszewski and Corcoran, 2011). However, oxidation can also be measured in other polymers such as PS or SBR (Yousif and Haddad, 2013).The ratio obtained with the introduction of these polymers were more similar to those found in lakes. This is an indication that few samples were highly oxidized, which can be related to the low sunshine hours in the Antuã river.

Other types of polymers were also found in both water and sediment samples, including polymers ranging from low to high specific density (0.88-2.2 g $cm^{-3}$). This high microplastic diversity may be related to sample collection applied in this study, to strong hydrodynamic conditions observed during sample collection, to heavy seasonal conditions (rain and wind) and to application of zinc chloride during sample processing (density: 1.5-1.6 g $cm^{-3}$). Rain and wind could cause direct inputs of MPs from the shoreline and surrounding terrestrial areas (Fischer et al., 2016).Other factors, such as intensive human activities, could also increase the diversity, since more diverse sources of inputs of waste plastic are associated (Zhang et al., 2017). Ballent et al. (2016) also found a high diversity of polymers in sediments using SPT (density: 1.5 g $cm^{-3}$) in density separation. In spite of having a higher efficiency in isolation of MPs, high-density reagents are mostly used in sediments (e.g. Ballent et al., 2016; Imhof et al., 2012; Mintenig, 2014; Nuelle et al., 2014; Zobkov and Esiukova, 2017), while in water samples analysis sodium chloride is the preference (e.g. Browne et al., 2011, 2010; Claessens et al., 2011; Mani et

al., 2015; Thompson et al., 2004).These results underline the importance of using an appropriate method of isolation of MPs in sediment as well as in water in order not to underestimate the abundance and polymer type found. Moreover, the presence of polymer PET (fibers) in Aguincheira is expected since the sampling site was close to a WWTP. According to McCormick et al. (2014) fibers from synthetic textiles such as polyesters were one of the main products associated to WWTP. They can be emitted through washing processes and are not completely removed by sewage treatment (Browne et al., 2011).Thus, a large abundance of fibers was expected mostly in Aguincheira and the results, especially for water samples, showed that pattern in both seasons. Fibers are considered by many authors the most abundant type in water, followed by fragments (Baldwin et al., 2016; Dris et al., 2015a; Fischer et al., 2016; Leslie et al., 2017; McCormick et al., 2014; Vermaire et al., 2017). In this study, among sediments samples, fragments were the most common particle found as in many other studies (Ballent et al., 2016; Horton et al., 2017; Vianello et al., 2013). Fragments were mainly PE and PP. These polymers are widely used in food packaging, milk boxes, supermarket bag and liquid yogurt boxes. In addition, among water samples fibers and foam were the most abundant type in March and in October respectively. The same pattern was also observed by Dris et al. (2015a) and Moore et al. (2011), whom, in April and May 2014, found mostly fibers and in November 2004 mostly foams, respectively. Foams were mainly expanded PS, which are widely used in buildings as insulating material or in packaging (PlasticsEurope, 2016).

The colored particles were the most dominant, followed by white, black and transparent. Among colored particles, the blue and green appear in higher concentrations. Blue particles were the most common color collected in stomachs of bluegill (*Lepomis macrochirus*) and longear (*Lepomis megalotis*) sunfish (Centrarchidae) (Peters and Bratton, 2016). In addition, white and transparent items were more abundant in Asian clams (*Corbicula fluminea*) (Su et al., 2016).

## 5. Conclusion

The concerning about MPs' contamination and its effects in aquatic systems have increased, however there are still immense gaps of knowledge regarding this problematic, especially in Portugal. This study provides new insights into MPs abundances and distribution in river water and sediments. It shows that Antuã river is severely influenced by MPs, in orders similar to that found in marine environment. Spatial and temporal distributions show substantial differences according to weather, population density and flow velocity. Moreover, this study emphasizes the importance of rivers as potential carriage systems of MPs. Since Portugal is the 12$^{th}$ country in Europe with the highest plastics demand (≈ 1 million tonnes) and 10% - 50% of plastic go to landfills (PlasticsEurope, 2016), it is urgent to monitor freshwater systems. Further studies should be performed and identified point sources to mitigate the MPs contamination. However, unified methods for sample collection, processing, characterisation and quantification (units) should be used.

## 6. References


Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L., Rennie, M.D., 2017. Microplastic contamination in Lake Winnipeg, Canada. Environ. Pollut. 225, 223–231. doi:10.1016/j.envpol.2017.02.072

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. doi:10.1016/j.marpolbul.2011.05.030

Arthur, C., Baker, J., 2011. Proceedings of the Second Research Workshop on Microplastic Marine Debris. November 5-6, 2010. NOAA Technical Memorandum NOS-OR&R-39.

Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of *Hyalella azteca* to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34, 2564–2572. doi:10.1002/etc.3093

Baldwin, A.K., Corsi, S.R., Mason, S.A., 2016. Plastic Debris in 29 Great Lakes Tributaries: Relations to Watershed Attributes and Hydrology. Environ. Sci. Technol. 50, 10377–10385. doi:10.1021/acs.est.6b02917

Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A., Longstaffe, F.J., 2016. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Mar. Pollut. Bull. 110, 383–395. doi:10.1016/j.marpolbul.2016.06.037

Barnes, D.K. a, Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. 364, 1985–1998. doi:10.1098/rstb.2008.0205

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47, 593–600. doi:10.1021/es302763x

Beyler, C.L., Hirschler, M.M., 2001. Thermal Decomposition of Polymers, in: DiNenno, P.J. (Ed.), SPE Handbook of Fire Protection Engineering. pp. 110–131. doi:10.1021/cm200949v

Biginagwa, F.J., Mayoma, B.S., Shashoua, Y., Syberg, K., Khan, F.R., 2016. First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia. J. Great Lakes Res. 42, 146–149. doi:10.1016/j.jglr.2015.10.012

Blair, R.M., Waldron, S., Phoenix, V., Gauchotte-Lindsay, C., 2017. Micro- and Nanoplastic Pollution of Freshwater and Wastewater Treatment Systems. Springer Sci. Rev. doi:10.1007/s40362-017-0044-7

Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. Environ. Sci. Pollut. Res. 23, 23522–23532. doi:10.1007/s11356-016-7584-2

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. Environ. Sci. Technol. 45, 9175–9179. doi:10.1021/es201811s

Browne, M.A., Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris along estuarine shorelines. Environ. Sci. Technol. 44, 3404–3409. doi:10.1021/es903784e

Carson, H.S., Nerheim, M.S., Carroll, K.A., Eriksen, M., 2013. The plastic-

associated microorganisms of the North Pacific Gyre. Mar. Pollut. Bull. 75, 126–132. doi:10.1016/j.marpolbul.2013.07.054

Castañeda, R.A., Avlijas, S., Simard, M.A., Ricciardi, A., Smith, R., 2014. Microplastic pollution in St. Lawrence River sediments. Can. J. Fish. Aquat. Sci. 71, 1767–1771. doi:10.1139/cjfas-2014-0281

Cerqueira, M.A., Silva, J.F., 2005. Monitorização da Qualidade da Água do Rio Antuã 1–8.

Claessens, M., Meester, S. De, Landuyt, L. Van, Clerck, K. De, Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62, 2199–2204. doi:10.1016/j.marpolbul.2011.06.030

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic Ingestion by Zooplankton. Environ. Sci. Technol. 47, 6646–6655. doi:10.1021/es400663f

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull. 62, 2588–2597. doi:10.1016/j.marpolbul.2011.09.025

Corcoran, P.L., Norris, T., Ceccanese, T., Walzak, M.J., Helm, P.A., Marvin, C.H., 2015. Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. Environ. Pollut. 204, 17–25. doi:10.1016/j.envpol.2015.04.009

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. doi:10.1016/j.envpol.2014.10.026

Dias, J.M., Lopes, J., Dekeyser, I., 1999. Hydrological characterisation of Ria de Aveiro, Portugal, in early summer. Oceanol. Acta 22, 473–485. doi:10.1016/S0399-1784(00)87681-1

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015a. Microplastic contamination in an urban area: a case study in Greater Paris. Environ. Chem. 12, 592. doi:10.1071/EN14167

Dris, R., Imhof, H., Sanchez, W., Gasperi, J., Galgani, F., Tassin, B., Laforsch, C., 2015b. Beyond the ocean: contamination of freshwater ecosystems with (micro-)plastic particles. Environ. Chem. 12, 539. doi:10.1071/EN14172

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28, 2. doi:10.1186/s12302-015-0069-y

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82. doi:10.1016/j.watres.2015.02.012

Eisma, D., Cadeé, G.C., 1991. Particulate Matter Processes in Estuaries, in: Degens, E.T., Kempe, S., Richey, J.E. (Eds.), Biogeochemistry of Major World Rivers. John Wiley and Sons Ltd, pp. 283–296.

Epa, 1992. Plastic Pellets in the Aquatic Environment: Sources and Recommendations, United States Environmental Protection Agency. Office of Water, Washington DC. doi:EPA 842-S-93-001

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. 77, 177–182. doi:10.1016/j.marpolbul.2013.10.007

Eubeler, J.P., Bernhard, M., Knepper, T.P., 2010. Environmental biodegradation of synthetic polymers II. Biodegradation of different polymer groups. TrAC Trends Anal. Chem. 29, 84–100. doi:10.1016/j.trac.2009.09.005

Faure, F., Demars, C., Wieser, O., Kunz, M., de Alencastro, L.F., 2015. Plastic pollution in Swiss surface waters: nature and concentrations, interaction with pollutants. Environ. Chem. 12, 582. doi:10.1071/EN14218

Fischer, E.K., Paglialonga, L., Czech, E., Tamminga, M., 2016. Microplastic pollution in lakes and lake shoreline sediments – A case study on Lake Bolsena and Lake Chiusi (central Italy). Environ. Pollut. 213, 648–657. doi:10.1016/j.envpol.2016.03.012

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake.

Mar. Pollut. Bull. 85, 156–163. doi:10.1016/j.marpolbul.2014.06.001

Frias, J.P.G.L., Antunes, J.C., Sobral, P., 2013. Local marine litter survey - A case study in Alcobaça municipality, Portugal. Rev. Gestão Costeira Integr. 13, 169–179. doi:10.5894/rgci395

Frias, J.P.G.L., Gago, J., Otero, V., Sobral, P., 2016. Microplastics in coastal sediments from Southern Portuguese shelf waters. Mar. Environ. Res. 114, 24–30. doi:10.1016/j.marenvres.2015.12.006

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1, 116. doi:10.1038/s41559-017-0116

GESAMP, 2010. Proceedings of the GESAMP International Workshop on micro-plastic particles as a vector in transporting persistent, bio-accumulating and toxic substances in the oceans., in: Bowmer, T., Kershaw, P. (Eds.), . p. 68. doi:10.1007/978-94-011-3610-5

Hidalgo-ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environ. Sci. Technol. 46, 3060−3075. doi:dx.doi.org/10.1021/es2031505

Hoellein, T., Rojas, M., Pink, A., Gasior, J., Kelly, J., 2014. Anthropogenic Litter in Urban Freshwater Ecosystems: Distribution and Microbial Interactions. PLoS One 9, e98485. doi:10.1371/journal.pone.0098485

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141. doi:10.1016/j.scitotenv.2017.01.190

Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. Curr. Biol. 23, R867–R868. doi:10.1016/j.cub.2013.09.001

Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P., Laforsch, C., 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnol. Oceanogr. Methods 10, 524–537. doi:10.4319/lom.2012.10.524

INE, 2015a. Anuário estatistico da Região Centro. Lisboa, Portugal.

INE, 2015b. Anuário Estatístico da Região do Norte. Lisboa, Portugal.

Kessarkar, P.M., Purnachandra Rao, V., Shynu, R., Mehra, P., Viegas, B.E., 2010. The Nature and Distribution of Particulate Matter in the Mandovi Estuary, Central West Coast of India. Estuaries and Coasts 33, 30–44. doi:10.1007/s12237-009-9226-0

Klein, S., Worch, E., Knepper, T.P., 2015. Occurrence and Spatial Distribution of Microplastics in River Shore Sediments of the Rhine-Main Area in Germany. Environ. Sci. Technol. 49, 6070–6076. doi:10.1021/acs.est.5b00492

Laglbauer, B.J.L., Franco-Santos, R.M., Andreu-Cazenave, M., Brunelli, L., Papadatou, M., Palatinus, A., Grego, M., Deprez, T., 2014. Macrodebris and microplastics from beaches in Slovenia. Mar. Pollut. Bull. 89, 356–366. doi:10.1016/j.marpolbul.2014.09.036

Lambert, S., Sinclair, C., Boxall, A., 2014. Occurence, Degradation and Effect of Polymer-Based Materials in the Environment. Rev. Environ. Contam. Toxicol. 227, 1–53. doi:10.1007/978-3-319-01327-5_1

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., Schludermann, E., 2014. The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ. Pollut. 188, 177–181. doi:10.1016/j.envpol.2014.02.006

Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environ. Int. 101, 133–142. doi:10.1016/j.envint.2017.01.018

Löder, M.G.J., Gerdts, G., 2015. Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal, in: Bergmann, M., Gutow, L., Klages, M. (Ed.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 201–227. doi:10.1007/978-3-319-16510-3_8

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (*Danio rerio*) and Toxic Effects in Liver. Environ. Sci. Technol. 50, 4054–4060. doi:10.1021/acs.est.6b00183

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., Nava-Saucedo, J.-E., 2008. Polymer biodegradation: Mechanisms and estimation techniques – A review. Chemosphere 73, 429–442. doi:10.1016/j.chemosphere.2008.06.064

Mani, T., Hauk, A., Walter, U., Burkhardt-Holm, P., 2015. Microplastics profile along the Rhine River. Sci. Rep. 5, 17988. doi:10.1038/srep17988

Martins, J., Sobral, P., 2011. Plastic marine debris on the Portuguese coastline: A matter of size? Mar. Pollut. Bull. 62, 2649–2653. doi:10.1016/j.marpolbul.2011.09.028

Masura, J., Baker, J., Foster, G., Arthur, C., 2015. Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments., NOAA Technical Memorandum NOS-OR&R-48.

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River. Environ. Sci. Technol. 48, 11863–11871. doi:10.1021/es503610r

McKeen, L.W., 2013. The effect of UV light and weather on plastics and elastomers, 3rd ed. William Andrew: Norwich, NY, USA.

Milliman, J.D., Huang-ting, S., Zuo-sheng, Y., H. Mead, R., 1985. Transport and deposition of river sediment in the Changjiang estuary and adjacent continental shelf. Cont. Shelf Res. 4, 37–45. doi:10.1016/0278-4343(85)90020-2

Mintenig, S., 2014. Planktonic Microplastic in the North Sea - A new extraction method for the detection by Fourier Transform Infrared Spectroscopy (FTIR). Master Thesis. Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany.

Moatar, F., Person, G., Meybeck, M., Coynel, A., Etcheber, H., Crouzet, P., 2006. The influence of contrasting suspended particulate matter transport regimes on the bias and precision of flux estimates. Sci. Total Environ. 370, 515–531. doi:10.1016/j.scitotenv.2006.07.029

Moore, C.J., Lattin, G.L., Zellers, A.F., 2011. Quantity and type of plastic debris flowing from two urban rivers to coastal waters and beaches of Southern California. Rev. Gestão Costeira Integr. 11, 65–73. doi:10.5894/rgci194

Moreno, F.S.B.A., 2000. Estudo dos mecanismos de dispersão de elementos vestigiais em sedimentos de correntes e águas superficiais da bacia hidrográfica do rio Antuã. PhD thesis. University of Aveiro, Aveiro, Portugal.

Naden, P.S., Murphy, J.F., Old, G.H., Newman, J., Scarlett, P., Harman, M., Duerdoth, C.P., Hawczak, A., Pretty, J.L., Arnold, A., Laizé, C., Hornby, D.D., Collins, A.L., Sear, D.A., Jones, J.I., 2016. Understanding the controls on deposited fine sediment in the streams of agricultural catchments. Sci. Total Environ. 547, 366–381. doi:10.1016/j.scitotenv.2015.12.079

Nuelle, M.-T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 184, 161–169. doi:10.1016/j.envpol.2013.07.027

Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indic. 34, 641–647. doi:10.1016/j.ecolind.2013.06.019

Peters, C.A., Bratton, S.P., 2016. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. Environ. Pollut. 210, 380–387. doi:10.1016/j.envpol.2016.01.018

PlasticsEurope, 2016. Plastics - the Facts 2016: An analysis of European plastics production, demand and waste data 1–37.

Rech, S., Macaya-Caquilpán, V., Pantoja, J.F., Rivadeneira, M.M., Jofre Madariaga, D., Thiel, M., 2014. Rivers as a source of marine litter – A study from the SE Pacific. Mar. Pollut. Bull. 82, 66–75. doi:10.1016/j.marpolbul.2014.03.019

Reddy, M.M., Gupta, R.K., Gupta, R.K., Bhattacharya, S.N., Parthasarathy, R., 2008. Abiotic Oxidation Studies of Oxo-biodegradable Polyethylene. J. Polym. Environ. 16, 27–34. doi:10.1007/s10924-008-0081-z

Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends Anal. Chem. 65, 47–53. doi:10.1016/j.trac.2014.10.011

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263.

doi:10.1038/srep03263

Ryan, P.G., Moore, C.J., van Franeker, J. a, Moloney, C.L., 2009. Monitoring the abundance of plastic debris in the marine environment. Philos. Trans. R. Soc. B Biol. Sci. 364, 1999–2012. doi:10.1098/rstb.2008.0207

Schindler, D.E., Scheuerell, M.D., 2002. Habitat coupling in lake ecosystems. Oikos 98, 177–189. doi:10.1034/j.1600-0706.2002.980201.x

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83. doi:10.1016/j.envpol.2013.10.013

Simpson, B.S.L., Batley, G.E., Chariton, A.A., Stauber, J.L., King, C.K., Chapman, J.C., Hyne, R. V, Gale, S.A., Roach, A.C., Maher, W.A., Simpson, S.L., 2005. Handbook for Sediment Quality Assessment Quality Assessment. CSIRO, Bangor, NSW.

Song, Y.K., Hong, S.H., Jang, M., Kang, J.-H., Kwon, O.Y., Han, G.M., Shim, W.J., 2014. Large Accumulation of Micro-sized Synthetic Polymer Particles in the Sea Surface Microlayer. Environ. Sci. Technol. 48, 9014–9021. doi:10.1021/es501757s

Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in Taihu Lake, China. Environ. Pollut. 216, 711–719. doi:10.1016/j.envpol.2016.06.036

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838. doi:10.1126/science.1094559

Urgert, W., 2015. Microplastics in the rivers Meuse and Rhine. Master thesis. Open University of the Netherlands, Heerlen, Netherlands.

Vermaire, J.C., Pomeroy, C., Herczegh, S.M., Haggart, O., 2017. Microplastic abundance and distribution in the open water and sediment of the Ottawa River, Canada, and its tributaries. FACETS 2, 301–314. doi:10.1139/facets-2016-0070

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuar. Coast.

Shelf Sci. 130, 54–61. doi:10.1016/j.ecss.2013.03.022

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 12. doi:10.1186/s12302-014-0012-7

Walling, D.E., 2009. The Impact of Global Change on Erosion and Sediment Transport by Rivers: Current Progress and Future Challenges 81, 30. doi:978-92-3-104135-8

Wang, J., Peng, J., Tan, Z., Gao, Y., Zhan, Z., Chen, Q., Cai, L., 2017. Microplastics in the surface sediments from the Beijiang River littoral zone: Composition, abundance, surface textures and interaction with heavy metals. Chemosphere 171, 248–258. doi:10.1016/j.chemosphere.2016.12.074

Wang, W., Wang, Q., Dang, W., 2009. Durability of a Rice-Hull--Polyethylene Composite Property Change After Exposed to UV Weathering. J. Reinf. Plast. Compos. 28, 1813–1822. doi:10.1177/0731684408090574

Yousif, E., Haddad, R., 2013. Photodegradation and photostabilization of polymers, especially polystyrene: review. Springerplus 2, 398. doi:10.1186/2193-1801-2-398

Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., Edgeworth, M., Gałuszka, A., Jeandel, C., Leinfelder, R., McNeill, J.R., Steffen, W., Summerhayes, C., Wagreich, M., Williams, M., Wolfe, A.P., Yonan, Y., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4–17. doi:10.1016/j.ancene.2016.01.002

Zbyszewski, M., Corcoran, P.L., 2011. Distribution and Degradation of Fresh Water Plastic Particles Along the Beaches of Lake Huron, Canada. Water, Air, Soil Pollut. 220, 365–372. doi:10.1007/s11270-011-0760-6

Zbyszewski, M., Corcoran, P.L., Hockin, A., 2014. Comparison of the distribution and degradation of plastic debris along shorelines of the Great Lakes, North America. J. Great Lakes Res. 40, 288–299. doi:10.1016/j.jglr.2014.02.012

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “Plastisphere”:

Microbial Communities on Plastic Marine Debris. Environ. Sci. Technol. 47, 7137–7146. doi:10.1021/es401288x

Zhang, K., Xiong, X., Hu, H., Wu, C., Bi, Y., Wu, Y., Zhou, B., Lam, P.K.S., Liu, J., 2017. Occurrence and Characteristics of Microplastic Pollution in Xiangxi Bay of Three Gorges Reservoir, China. Environ. Sci. Technol. 51, 3794–3801. doi:10.1021/acs.est.7b00369

Zobkov, M., Esiukova, E., 2017. Microplastics in Baltic bottom sediments: Quantification procedures and first results. Mar. Pollut. Bull. 114, 724–732. doi:10.1016/j.marpolbul.2016.10.060

# Chapter 4
# General discussion

## Chapter 4 - General discussion

This study provides new insights into standardization of methodologies in water samples as well as into microplastics (MPs) abundances and distribution in a Portuguese river. According to cost-effective criteria, MPs separation, one of the most questionable procedure (Cole et al., 2014; Hidalgo-ruz et al., 2012; Masura et al., 2015; Qiu et al., 2016; Tagg et al., 2015), should be performed using hydrogen peroxide with addition of zinc chloride. Zinc chloride, isolated or together with a reagent of organic matter degradation, were the most efficient method reaching more than 90% of MPs' recovery. This compound can reach densities around 1.5-1.6 g $cm^{-3}$ including a wide range of polymers such as PE, PP, PET and PVC (type of polymers used in this study) (Claessens et al., 2013; Imhof et al., 2012; Liebezeit and Dubaish, 2012; Mintenig, 2014; Nuelle et al., 2014; Van Cauwenberghe et al., 2015; Zobkov and Esiukova, 2017). These polymers with higher density than freshwater (≈ 1 g $cm^{-3}$) are usually submerged, however physical factors, seasonal conditions, human activities or other particle's characteristics could influence the fate of polymer in aquatic environment (Kessarkar et al., 2010; Moatar et al., 2006; Rocha-Santos and Duarte, 2015; Simpson et al., 2005). Mainly, physical factors, such as flow velocity, river morphology, turbulence and seasonal variability of water flows, have an important role in the availability of these particles in water. Thus, zinc chloride is the adequate method for a better estimation of abundance and polymer type of MPs in water as well as in sediment. However, the density separation methods isolated made handling and examination of the samples more difficult, since they do not eliminate organic matter. The procedure could be complemented with a reagent of organic matter degradation such as hydrogen peroxide (WPO). Hydrogen peroxide allowed a better observation of membrane filters and, consequently, an easily MPs' recovery. Although the application of this oxidizer is highly recommended for elimination of organic matter, it should not be applied routinely (Blair et al., 2017; Imhof et al., 2012; Liebezeit and Dubaish, 2012; Nuelle et al., 2014; Tagg et al., 2015).

Moreover, this methodology (hydrogen peroxide with addition of zinc chloride) was applied in both type of samples of Antuã river allowing an adequate

MPs' characterization and quantification. Most type of polymers identified in this study were low-density polymers such as PE and PP (67.6%) whereas 32.4% were high-density polymers (PET, PEA, PVA, PMMA, PFTE, Cellulose acetate). These two low-density polymers, PE and PP, are the two most produced polymers covering 48.5% of the European plastic demand. This, allied with its specific characteristics allow to a high widespread distribution in aqueous systems (Klein et al., 2015). Concerning the high-density polymers found, they represent around 26.2% of the European plastic demand (PlasticsEurope, 2016), an important percentage of the MPs that could be present in environment. These polymers were equally found in water and sediment samples showing the importance of using zinc chloride as a density separator. Hence, this study underlines the importance of rivers as sink of MPs that can potential be carriage to marine systems. Furthermore, it highlights the urgent need to characterize freshwater systems in terms of diversity and quantity of MPs, as well as to identify potential sources to mitigate the MPs contamination.

In future studies, to assess the temporal variation of MPs it is important to extend the monitoring to contrasting seasons (wet and dry). Likewise, to better identify the river stretches more contaminated by MPs and to help localize the sources, it is recommended to include a reasonable number of sampling points, from the river source to its mouth. Moreover, knowing that MPs could affect the aquatic life, in a further step it is important to quantify MPs in organisms and evaluate their effects in species from distinct trophic and functional levels.

## 2. References

Blair, R.M., Waldron, S., Phoenix, V., Gauchotte-Lindsay, C., 2017. Micro- and Nanoplastic Pollution of Freshwater and Wastewater Treatment Systems. Springer Sci. Rev. doi:10.1007/s40362-017-0044-7

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. 70, 227–233. doi:10.1016/j.marpolbul.2013.03.009

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S.,

2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 4528. doi:10.1038/srep04528

Hidalgo-ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environ. Sci. Technol. 46, 3060−3075. doi:dx.doi.org/10.1021/es2031505

Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P., Laforsch, C., 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnol. Oceanogr. Methods 10, 524–537. doi:10.4319/lom.2012.10.524

Kessarkar, P.M., Purnachandra Rao, V., Shynu, R., Mehra, P., Viegas, B.E., 2010. The Nature and Distribution of Particulate Matter in the Mandovi Estuary, Central West Coast of India. Estuaries and Coasts 33, 30–44. doi:10.1007/s12237-009-9226-0

Klein, S., Worch, E., Knepper, T.P., 2015. Occurrence and Spatial Distribution of Microplastics in River Shore Sediments of the Rhine-Main Area in Germany. Environ. Sci. Technol. 49, 6070–6076. doi:10.1021/acs.est.5b00492

Liebezeit, G., Dubaish, F., 2012. Microplastics in Beaches of the East Frisian Islands Spiekeroog and Kachelotplate. Bull. Environ. Contam. Toxicol. 89, 213–217. doi:10.1007/s00128-012-0642-7

Masura, J., Baker, J., Foster, G., Arthur, C., 2015. Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments., NOAA Technical Memorandum NOS-OR&R-48.

Mintenig, S., 2014. Planktonic Microplastic in the North Sea - A new extraction method for the detection by Fourier Transform Infrared Spectroscopy (FTIR). Master Thesis. Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany.

Moatar, F., Person, G., Meybeck, M., Coynel, A., Etcheber, H., Crouzet, P., 2006. The influence of contrasting suspended particulate matter transport regimes on the bias and precision of flux estimates. Sci. Total Environ. 370, 515–531. doi:10.1016/j.scitotenv.2006.07.029

Nuelle, M.-T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 184, 161–169. doi:10.1016/j.envpol.2013.07.027

PlasticsEurope, 2016. Plastics - the Facts 2016: An analysis of European plastics production, demand and waste data. Brussels.

Qiu, Q., Tan, Z., Wang, J., Peng, J., Li, M., Zhan, Z., 2016. Extraction, enumeration and identification methods for monitoring microplastics in the environment. Estuar. Coast. Shelf Sci. 176, 102–109. doi:10.1016/j.ecss.2016.04.012

Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends Anal. Chem. 65, 47–53. doi:10.1016/j.trac.2014.10.011

Simpson, B.S.L., Batley, G.E., Chariton, A.A., Stauber, J.L., King, C.K., Chapman, J.C., Hyne, R. V, Gale, S.A., Roach, A.C., Maher, W.A., Simpson, S.L., 2005. Handbook for Sediment Quality Assessment Quality Assessment. CSIRO, Bangor, NSW.

Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging. Anal. Chem. 87, 6032–6040. doi:10.1021/acs.analchem.5b00495

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015. Microplastics in sediments: A review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17. doi:10.1016/j.marenvres.2015.06.007

Zobkov, M., Esiukova, E., 2017. Microplastics in Baltic bottom sediments: Quantification procedures and first results. Mar. Pollut. Bull. 114, 724–732. doi:10.1016/j.marpolbul.2016.10.060

# Appendices

## Appendices

## Appendix A

Figures 1 to 11 are the spectra obtained in the beginning of the study, being considered as blank or reference spectra of each type of plastic and its respective polymer.

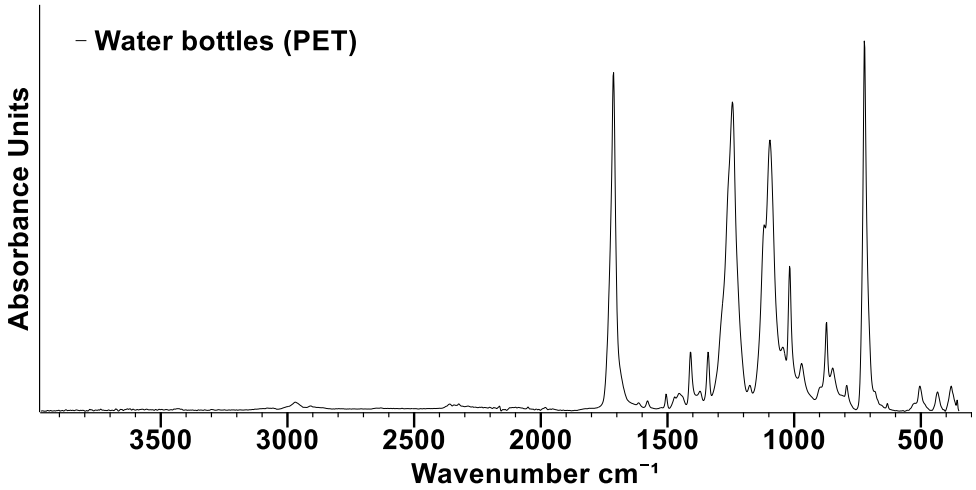


**Figure 1** – Plastic bottles – polymer PET.

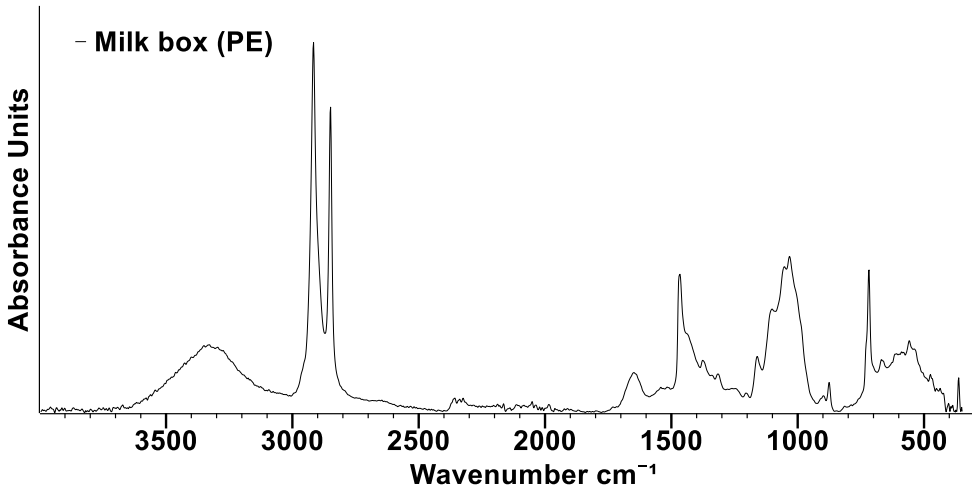


**Figure 2** – Tetra Pak - polymer PE.

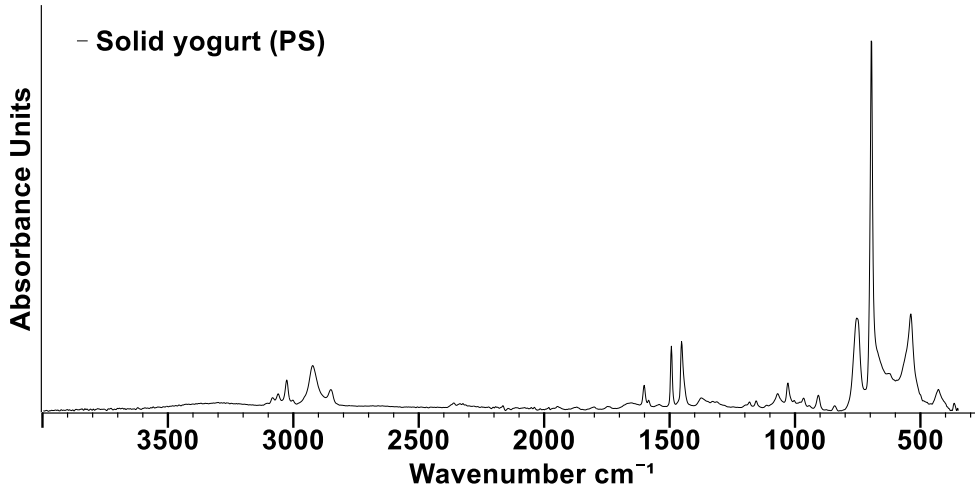


**Figure 3** – Solid yogurt – polymer PS.

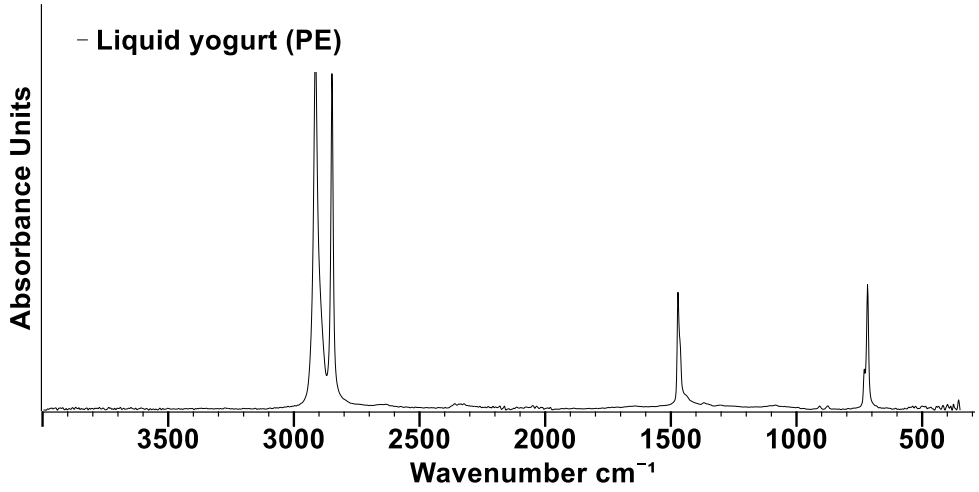


**Figure 4** – Liquid yogurt – polymer HDPE.

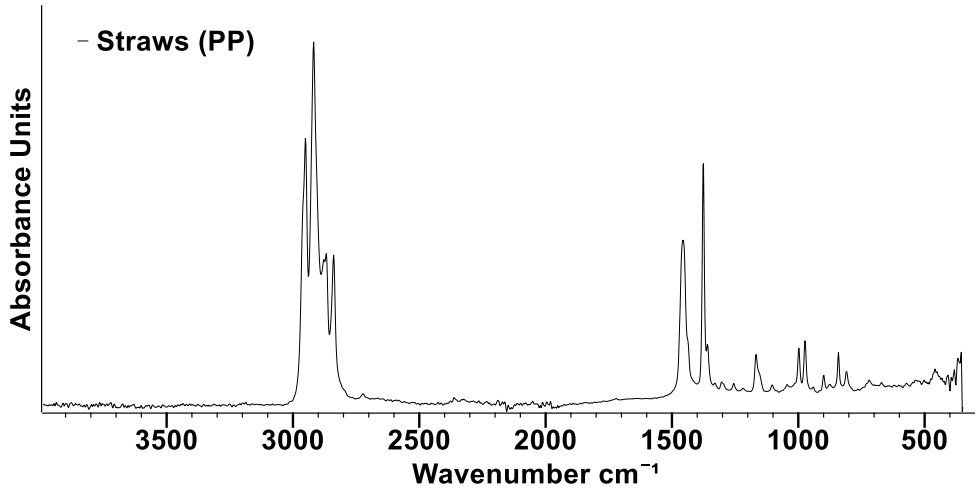


**Figure 5** – Straws – polymer PP.

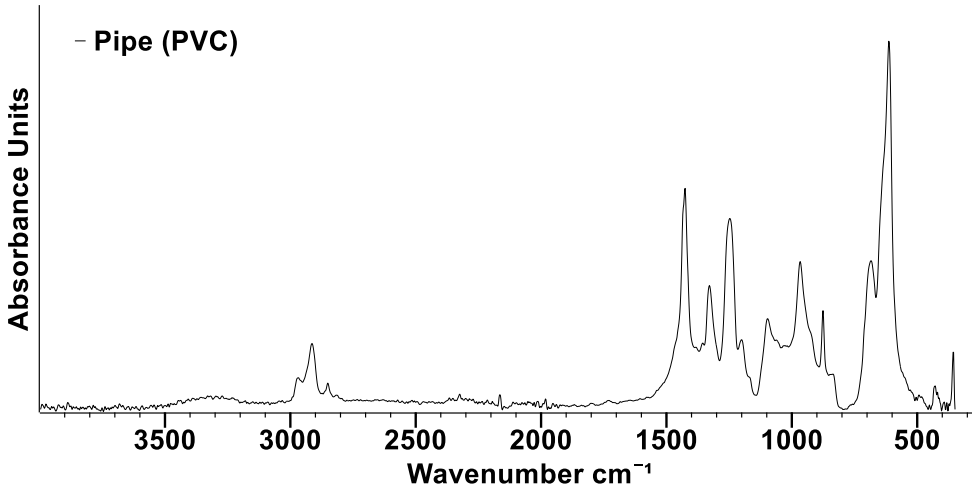


**Figure 6** – Pipe – polymer PVC.

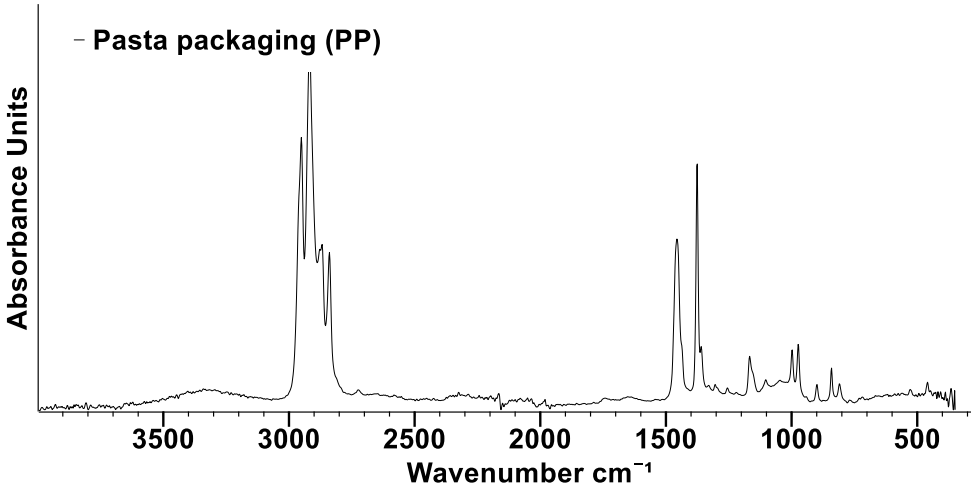


**Figure 7** – Pasta packaging – polymer PP.

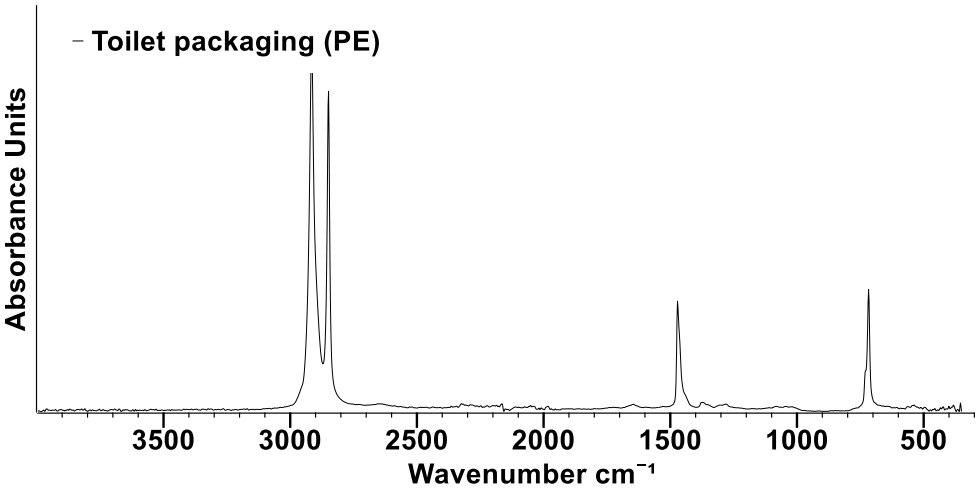


**Figure 8** – Toilet packaging – polymer PE.

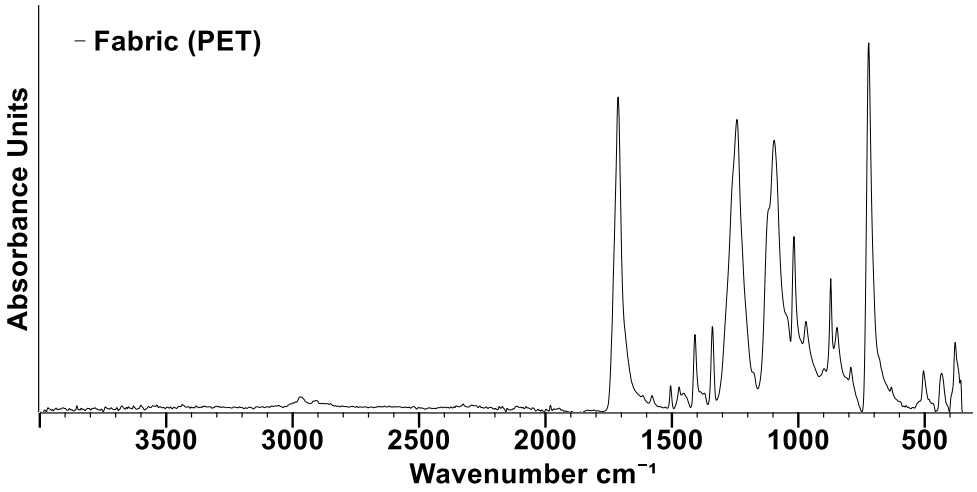


**Figure 9** – Fabric - polymer PET.

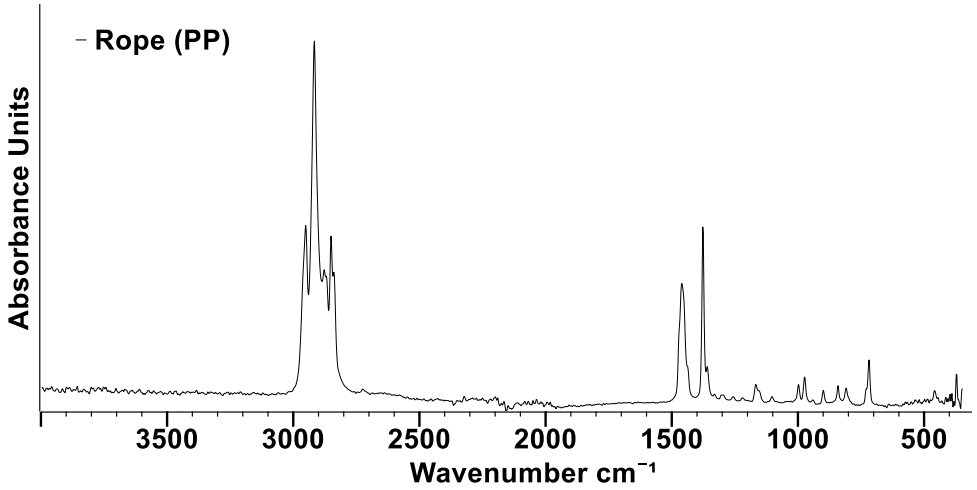


**Figure 10** – Rope - polymer PP.

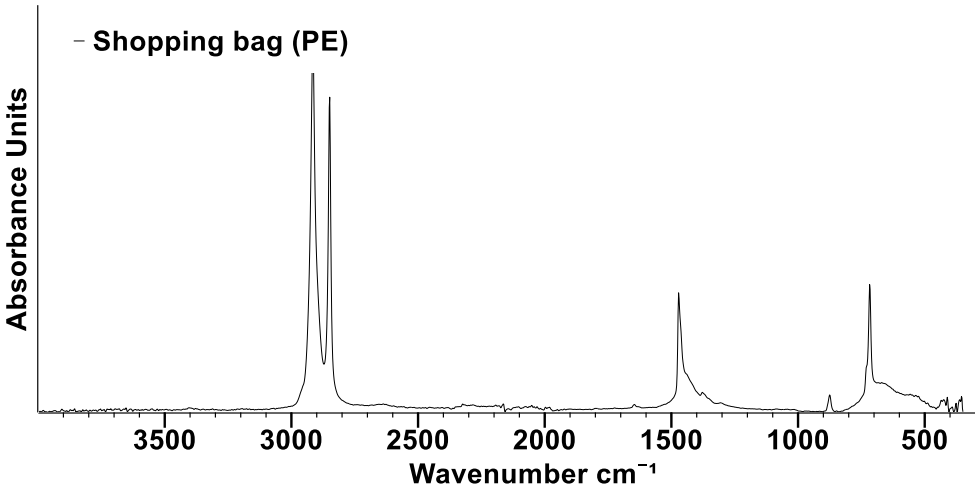


**Figure 11** – Shopping bag – polymer LDPE.

## Appendix B

Additional information of water and sediment samples during sampling collection and processing.

**Table 1 –** Additional characteristics of sampling sites during collection.

| Location | | Coordinates | Flow velocity ($m\ s^{-1}$) | Chemical parameters | | | |
|---|---|---|---|---|---|---|---|
| | | | | Conductivity ($\mu S\ cm^{-1}$) | pH | Temp. (ºC) | $O_2$ ($mg\ L^{-1}$) / (%) |
| São João da Madeira | 1 | 40º53’55.08"N 8º28’57.23"W | 0.23 | 80 | 7.8 | 12 | 10.5/99.8 |
| | 4 | | 0.02 | 101.9 | 6.5 | 17.2 | 7.3/89 |
| Aguincheira | 2 | 40º51’13.50"N 8º29’42.23"W | 0.01 | 123.2 | 7 | 12 | 10.4/99.8 |
| | 5 | | 0 | 230 | 7 | 17.6 | 3.6/46 |
| Estarreja | 3 | 40º45’23.84"N 8º33’40.96"W | 0.03 | 121.7 | 7 | 12 | 8.6/99.8 |
| | 6 | | 0.09 | 166 | 7.1 | 17.6 | 63/77 |

**Table 2 –** Weight of sediment obtained after dry step at 90ºC during 2 days.

| Location | | Initial mass (kg) | Dry sediment (kg) |
|---|---|---|---|
| São João da Madeira | 1 | 0.5 | 0.371 |
| | 4 | 0.5 | 0.392 |
| Aguincheira | 2 | 0.5 | 0.285 |
| | 5 | 0.5 | 0.224 |
| Estarreja | 3 | 0.5 | 0.280 |
| | 6 | 0.5 | 0.392 |

## Appendix C

Characterization of polymers type and oxidation level for polyethylene, polypropylene (PE), polystyrene (PP) and styrene/butadiene copolymer (SBR).

**Table 3 –** Compositional characteristics of 43 sampled plastics from Antuã river, after FTIR identification.

| #Analysis | Sample | Shape | Colour | Polymer | Oxidation |
|---|---|---|---|---|---|
| 1 | W1 | Fragment | Blue | PE | Low |
| 2 | W2 | Fragment | White | PP | Low |
| 3 | W2 | Fiber | Red | PTFE | - |
| 4 | W3 | Fragment | Transparent | PE | Low |
| 5 | W3 | Fragment | Orange | Not plastic | - |
| 6 | W3 | Fragment | Black | Not plastic | - |
| 7 | W3 | Fragment | Pink | PE | Low |
| 8 | W3 | Fragment | Green | PP | High |
| 9 | W3 | Film | Brown | N.I. | - |
| 10 | W3 | Pellet | Yellow | N.I. | - |
| 11 | W4 | Foam | White | PS | Low |
| 12 | W4 | Foam | Brown | PS | High |
| 13 | W4 | Foam | Gold | N.I. | - |
| 14 | W4 | Foam | Red | EVA | - |
| 15 | W4 | Foam | Brown | Cellulose acetate | - |
| 16 | W4 | Film | Black | PE | - |
| 17 | W4 | Film | White | PE | - |
| 18 | W4 | Film | Green | PP | Medium |
| 19 | W4 | Film | Transparent | PEA | - |
| 20 | W5 | Fragment | Orange | PP | Low |
| 21 | W5 | Fragment | Green | PP | Low |
| 22 | W5 | Fragment | White | PP | Low |
| 23 | W5 | Film | Brown | PET | - |
| 24 | S2 | Fragment | Gold | N.I. | - |
| 25 | S2 | Fragment | Orange | N.I. | - |

| | | | | | |
|---|---|---|---|---|---|
| 26 | S2 | Fragment | Green | PVA | - |
| 27 | S2 | Fragment | Silver | PET | - |
| 28 | S2 | Fragment | Grey | PE | - |
| 29 | S2 | Fragment | Transparent | PP | Medium |
| 30 | S2 | Fiber | White | PET | - |
| 31 | S2 | Fiber | Black | PVA | - |
| 32 | S2 | Fiber | Pink | SBR | High |
| 33 | S2 | Film | Green | Not plastic | - |
| 34 | S3 | Fragment | Red | PVA | - |
| 35 | S3 | Fiber | White | PP | Medium |
| 36 | S4 | Fragment | White | PE | Medium |
| 37 | S4 | Foam | White | PS | Medium |
| 38 | S5 | Fragment | Pink | PMMA | - |
| 39 | S5 | Fragment | Green | PE | Medium |
| 40 | S5 | Fragment | Beige | PE | - |
| 41 | S5 | Fragment | Transparent | PE | - |
| 42 | S5 | Pellet | Black | N.I. | - |
| 43 | S5 | Pellet | Brown | PEA | - |

**Legend:**

EVA – Ethylene-vinyl acetate

N.I. – Not identified

PE – Polyethylene

PP – Polypropylene

PEA – Poly(ethylacrylate)

PET – Polyethylene terephthalate

PMMA – Polymethylmethaacrylate

PS – Polystyrene

PTFE – Polytetrafluorethylene

PVA – Polyvinyl acetate

SBR – Butadiene/styrene copolymer